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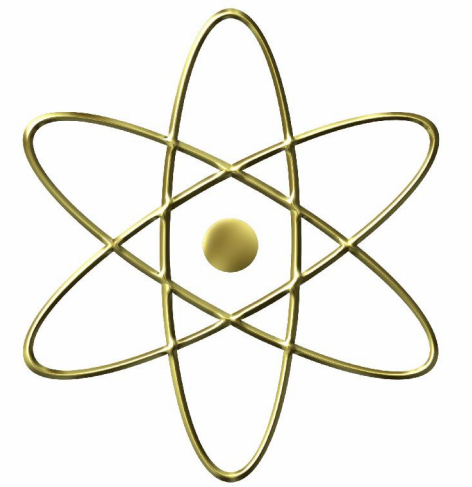

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1 **Automatic Generation of Extended ER Diagram Using Natural Language Processing**

Dr. Muhammad Shahbaz [1], Dr. Syed Ahsan [2], Muhammad Shaheen [3], Rao Muhammad Adeel Nawab[4], Syed Athar Masood [5]

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Abstract: Extended Entity Relationship Diagrams are an important step in information system design and software engineering. In the early seventies Peter Chen developed an efficient database management system, the ERD. Later on, ERD was enhanced to Extended ERD by adding new concepts like generalization and specialization. The inspiration of EERD emerged from the common need to many organizations to have a unified methodology for file structure and database design. To meet the demands of users, to interpret problem statements in English, applying all the rules and generating an EERD. The structural approach is used to parse the sentences and tag them into different parts of the speech. This is because a belief has been developed that semantics can be completely represented in structures. The structural approach is used to map the tagged words into entities, attributes and relationships. [Dr. Muhammad Shahbaz, Dr. Syed Ahsan, Muhammad Shaheen, Rao Muhammad Adeel Nawab, Syed Athar Masood. Automatic Generation of Extended ER Diagram Using Natural Language Processing. Journal of American Science 2011;7(8):1-10]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Geographic Information System (GIS), Usability, Interactivity, Human-GIS Interaction, Positional Accuracy, Hydrocarbon Exploration, Backpropogation Neural Network Full Text

2 **STYX : A CML based chem.-informatics facility**

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Abstract: Developing countries such as Pakistan lose out the benefits of global cheminformatics research unless immediate actions are taken to strengthen the infrastructure for their participation. They rely on dry labs because they don't have the wet labs. They need infrastructure so that cure can be found of the diseases and infections which attack the local human, animal and plant population as international pharmaceutical companies are less interested in these "poor man " diseases for lack of financial turnover. We feel that instead of supplying disease information and data to the foreign companies, we would then use this information to discover new drugs. [Syed Ahsan, Muhammad Shahbaz, Syed Athar Masood. STYX: A CML Based Chem-Informatics Facility. Journal of American Science 2011;7(8):11-12]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: STYX; CML based chem.-informatics facility Full Text

3 **Nurses' related factors influencing the use of physical restraint in critical care units**

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Abstract: Although physical restraints are used commonly to maintain the safety of critically ill patient, the use of physical restraints is associated with many adverse effects. Therefore, several attempts have been made to identify and control factors affecting restraint utilization in critical care units (CCUs). This study aims to identify nurses' related factors influencing the use of physical restraints in CCUs. This study was conducted in three of the CCUs of Alexandria Main University Hospital. Fifty critical care nurses, working in the above mentioned settings, who apply physical restraints, were recruited sequentially in this study. Fifty patients who were restrained and assigned to the observed nurses were included in this study. Patients who were restrained for a period of less than two hours were excluded. Two tools were used to collect the required data; nurses' restraint performance checklist, and nurses' related factors influencing the use of physical restraint questionnaire. It can be concluded from this study that older nurses and those with higher qualification and years of experience have better restraint related performance than others. Nurses' knowledge and performance are in need for improvement. Therefore, it is recommended to conduct in-service training programs for nurses working in CCUs on restraint utilization and restraint alternatives. The hospital should develop evidence based guideline on physical restraining to be available for all nurses and physicians in order to follow.
[Tarek H. Al-Khaled, Eman M. Zahran, and Azza H. El-Soussi. Nurses' related factors influencing the use of physical restraint in critical care units. Journal of American Science 2011; 7(8):13-22]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Nurse; factor; physical restraint; critical care unit Full Text

4 **Tracheal suctioning with versus without saline instillation**

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Abstract: The primary goal of tracheal suctioning to maintain a patent airway. It is considered as one of the most common procedure in critical care areas. Normal saline instillation into an artificial airway prior to suctioning is utilised by many health practitioners. However, there are conflicting views about its safety. This study was conducted in two phases. Phase "1" aims to determine how often normal saline is used during tracheal suctioning, and determine nurses and physicians' knowledge regarding advantages and disadvantages of normal saline instillation (INS) before suctioning. Phase "2" aims to compare between the effects of suctioning with saline versus suctioning without saline on a number of physiological response parameters. This study was conducted at Causality Care Unit, and General Intensive Care Unit (ICU), Main University Hospital, University of Alexandria; including;. Ninety two nurses and 16 physicians working in the previously mentioned settings were included in the first phase of the study, while 26 adult critically ill patients were included in the second phase of the study. Two tools were used to collect required data; tool (I) tracheal suctioning questionnaire used to collect data for phase "1" and tool (II) physiological response parameters assessment sheet used to collect data for phase "2". Based on the findings of this study, it can be concluded that nurses and physicians frequently use saline before suctioning. A considerable number of them recognize the most common advantages and disadvantages to the INS. In relation to the comparison between suctioning with and without INS, this study shows that INS carries out several risks, including; significant elevation in $PaCO_2$ immediately after suctioning and reduction in oxygen tension and saturation, 5 minutes after suctioning. So, nurses and physicians have to be aware on these disadvantages of INS. In addition, alternative measures facilitating liquefying secretion and its removal have to be utilized instead of INS.
[Eman M. Zahran and Ahmed A. Abd El-Razik. Tracheal suctioning with versus without saline instillation. Journal of American Science 2011;7(8):23-32]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: instillation of normal saline, suction, physiologic response to suction. Full Text

5 **Spatio-temporal Variations in Phytoplankton Biomass and diversity in a Tropical Eutrophic Lagoon, Nigeria**

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Abstract: Taxonomic inventorisation and spatio-temporal variations in the phytoplankton species biomass and diversity, in relation to environmental parameters at the Ologe lagoon, Lagos were investigated from February, 2002 to January, 2004. The annual rainfall, concentrated in one season, initiated increased total solids and nutrient values whereas low dissolved oxygen, conductivity, transparency, and cation concentrations were recorded. The phytoplankton species biomass, composition and water quality indices exhibited seasonal changes closely related to the pattern of rainfall. Estimation of phytoplankton biomass by cell count showed a range of 849 to 1771707 cells/ml with mean value of 44052cell/ml. The phytoplankton flora of the lagoon belonged to five main algal phyla, namely Bacillariophyta (84%), Cyanophyta (15.92%), Chlorophyta (0.06%), Euglenophyta (0.018%) and Prryophyta (0.002%). One hundred and nineteen species belonging to forty-nine genera were observed, with diatoms forming the most abundant and diverse. A total of forty-eight species belonging to eighteen genera was found in diatoms. This was followed by green algae, with thirty-two species from fourteen genera, Cyanobacteria, with twenty-three species from eleven genera, euglenoids with seventeen species from five genera, while the dinoflagelates had one species. Nine phytoplankton species were reported to be potentially harmful/toxic bloom species. 57 bio-indicator species were recorded during the period of study. With regard to existing checklist of phytoplankton species, 10 new species are the first reports for Lagos lagoon complex, south-western Nigeria.The centric diatom *Aulacoseira* and cyanobacterium *Microcystis* dominated the phytoplankton community spectrum and their dominance in the Lagoon in both seasons suggests a single floristic grouping. The observed range of bio-indicator species within Ologe lagoon showed that the lagoon is eutrophic. Co-efficient of similarity index indicated that stations close to each other are more similar, than stations further apart.
[Paul. Chuks. Onuoha, Dike Ikeagwu Nwankwo, Lucian Obinna Chukwu and [4]Vyverman, Wim. **Spatio-temporal Variations in Phytoplankton Biomass and diversity in a Tropical Eutrophic Lagoon, Nigeria.** Journal of American Science 2011;7(8):33-46]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: Seasonal changes, phytoplankton composition, cell number, bio-indicator, diversity, eutrophic, rainfall. Full Text

6 **Self-esteem and quality of life among visually impaired children in Assiut City, Egypt**

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Abstract: Nowadays, quality of life is one of the important aspect in programming and giving service to disabled and blindness is one of the most important and common kinds of physical handicaps. This study aimed to: determine Self-esteem & quality of life among visual impaired children in Assiut City. Descriptive cross sectional research design was used in this study. The studied children were 100 children from El-Noor School, Assiut City, 22 children from last two years of primary school, 44 children from preparatory school, 34 children from secondary school with age ranged from 12 to 18 years and both sex. This study is divided to four tools; the first tool was structured interview sheet included biosocial data of children, time and causes of disability, second tool to assess social class by using Abd-El-Twab scale 1998. The third tool - Self-esteem scale, it includes 10 items. , the final tool to assess quality of life data, it consists of 68 items. Following approval for the research, personal meetings were held with each child in school sitting. Data was collected during the period from first of September 2009 to the end of April 2010. Obtained results revealed that more than two-thirds of studied children aged between 12-18 years. The mean age of studied children is 15.87 ± 3.03 and more than half of them were males. Nearly three quarters of studied sample had low social class (72%). In addition; nearly half of children with high total quality of life score were come from high social class compared with 8.3% from low social class children with highly statistically significant difference. Also, it was found that 40% of studied children with high total quality of life scores were outdoor school residence compared to only 8.2% of indoor school residence (p -value 0.003). Further, more than half of studied children with low social class had low self-esteem compared to more than three - quarters of normal self - esteem children were come from high social class. 71.4% of normal self-esteem children were present in small family compared by two third of low self-esteem score were present in large family**.** It is concluded that children from high social class had higher quality of life and normal self esteem than other children**.** It is recommended that multicomponant intervention with a focus on the children and their families are needed to improve self esteem and quality of life of visually impaired children. Designing and implementing of a psychological counseling program for visually impaired children to improve their emotional statues and help them to coping effectively.
[Safaa A. M. . Kotb; Marzoka A. Gadallah and Salwa A. Marzouk. Self-esteem and quality of life among visually impaired children in Assiut City, Egypt. Journal of American Science 2011;7(8):47-57].(ISSN: 1545-1003). http://www.americanscience.org.

Key words: Visually impaired, Self-esteem, quality of life, school age children Full Text

7 **A Newly Developed Exercise Program for Treatment of Mechanical Low Back Pain Associated with Accentuated Lumbar Lordosis**

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Abstract: The relationship between low back pain and lumbar lordosis has been studied extensively with many contradictory conclusions. The already existing exercise programs to correct the accentuated lumbar lordosis have got many disadvantages. Objectives: Introduce newly developed exercise program to verify the relationship between mechanical low back pain and increased lumbar curve. A new program of exercise has been designed to alleviate the disadvantage of the previous programs. Study design: experimental, one group . Twelve patients with average age 39.3±4.5 years were included in the study, all have chronic low back pain over one year with lordotic angle over 50 degrees. Lateral X ray-films were taken before, after one and two months of treatment .A metal frame was designed to facilitate the use of plumb line, also a wedged pillow with an angle of 115˚ and stretch tool was designed for the execution of the exercise program. The patients did not receive any source of heat or medication throughout the treatment. The results revealed to a significant reduction of pelvic inclination, lumbosacral angle and lumbar curve were 5.4˚, 2.6˚ and 3.00˚ , respectively after the end of the second month of treatment . There is gradual and complete relief of pain without recurrence. It is concluded that this new program alleviated the disadvantages of the previous programs .It confirm the relation between low back pain and accentuated lumbar lordosis.
[Fatma A. El-Hamalawy. **A Newly Developed Exercise Program for Treatment of Mechanical Low Back Pain Associated with Accentuated Lumbar Lordosis**. Journal of American Science 2011;7(8):58-70].(ISSN: 1545-1003). http://www.americanscience.org.

Keywords: lordosis, lumbosacral angle, pelvic inclination, X-ray measurements, accentuated, correction, William's exercises, mechanical low back pain Full Text

8 **Forward Head Correction Exercises For Of Management Myogenic Tempromandibular Joint Dysfunction**

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Abstract: Objectives: To investigate the effect of independent forward head postural exercise on myogenic tempromandibular joint dysfunction. Methods :The study included fifteen females **,** diagnosed as myogenic TMJ dysfunction with limited mouth opening aged between 20-40 years(27.1± 4.6 years).TMJ pain was Persistent for at least six months(12.3±5.3) and Patients were observed to have a forward head posture .The measurements included vertical mouth opening measured in millimeters by Standard ruler, pain intensity using visual analogue scale and craniocervical posture on lateral cephalometic .Each patient received exercise program consisting of 1- strengthening exercise of deep cervical flexors and scapular retractors 2- stretching exercise of the suboccipital muscles and pectoralis muscles. Results: The result showed significant decrease in crainocervical angle and lower cervical curvature. Also there was a significant increase in vertical active mouth opening (P=0.000) and decreased pain level of masticatory system (P=0.000). The correlation between mouth opening and the Craniocervical angle was found to be significant .No significant correlation was found between upper and lower cervical. Independent forward head correction exercise program was found to be effective in improving myogenic TMJD and support the relation between forward head posture and TMJD.
[Fatma A. El-Hamalawy. **Forward Head Correction Exercises For Of Management Myogenic Tempromandibular Joint Dysfunction.** Journal of American Science 2011;7(8):71-77]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Myogenic; tempromandibulr joint dysfunction; myofascial pain; forward head posture; posture exercise. Full Text

9 **Inhibition Effect Of Some Cationic Gemini Surfactants For Carbon Steel In Sea Water**

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Abstrac Corrosion inhibition of carbon steel alloy in sea water by different synthesized gemini surfactants {12-2-12, 14-2-14 and 16-2-16} have been investigated using weight loss, potentiodynamic polarization and surface tension measurements. The data obtained from all the used methods are in good agreement with each other and ensure the excellent inhibition efficiency of the tested surfactants for carbon steel in sea water. The inhibition efficiency increases with increasing the concentration of the studied inhibitors. Also, the adsorption ability of the surfactant molecules on carbon steel surface increases with the increase in the hydrocarbon chain length of the surfactant molecule from 12 to 16 and through 14 C atoms; meanwhile, the isotherm of 16-2-16 declares the formation of multilayer onto the used metal surface. The inhibitive action of the studied surfactants follow the order: 16-2-16 > 14-2-14> 12-2-12. The morphological changes of carbon steel surface were studied by Scanning Electron Microscope [SEM].
[Nessim I .M. ; Hamdy A.; Osman M.M. and Shalaby M. N. **Inhibition Effect Of Some Cationic Gemini Surfactants For Carbon Steel In Sea Water.** Journal of American Science 2011; 7(8): 78-90]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Carbon steel, sea water, gemini surfactant, weight loss, potentiodynamic, adsorption onto Solid/liquid interface. Full Text

10 **Prophylactic and therapeutic evaluation of the phytobiotic (Orego-stim)® in chicken experimentally infected with *E. coli***

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Abstract: The prophylactic and therapeutic effects of the phytobiotic (Orego-stim)®was evaluated in chicken experimentally infected with Novobiocin marked *E. coli O78*. Enrofloxacin (Opitryl)® was used as a standard. The obtained results demonstrated that, birds prophylactically received Orego-stim® showed more favorable clinical signs, mortality rate, P.M. lesions, recovery rate, bacterial reisolation results and growth performance. Both cellular and humeral immunity were enhanced. A decrease in the mean values of serum ALT & AST ,albumin, uric acid and creatinine levels were recorded that may provide evidence for the hepato and renoprotective effects of the

essential oils. It could be concluded that, Orego-stim® can be considered a promising mixture of essential oils due to its high efficacy (growth performance, antibacterial and immunomodulating effects) and positive impact on both liver and kidney functions. The study highly recommends the use of Orego-stim® as a prophylactic agent in dealing with *E. coli* infection in chicken however, its concurrent administration with enrofloxacin in treatment of such case revealed the most favorable outcomes.
[Naglaa Z. H. Eleiwa; E. M. El Sayed and A. A. Nazim. **Prophylactic and therapeutic evaluation of the phytobiotic (Orego-stim)® in chicken experimentally infected with *E. coli*.** Journal of American Science 2011; 7(8):91-102]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: Orego-stim®, essential oils, *E. coli*, efficacy, side effects, chicken Full Text

11 **Assessment of female adolescent Reproductive health needs in Assiut city**

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Abstract: Adolescents around 17million in Egypt (2005) have different needs and require different counseling approaches and more information. Adolescence is a critical stage for risk-taking because adolescents are moving toward independence and tend to experiment and test limits**. Aim of this study**: was to determine the problems of female adolescents, and provide information about reproductive health. **Subject and methods**: Cross-sectional analytic study was conducted in nursing secondary schools and technical institutes in Assiut city in Upper Egypt with total number of 514 adolescent female students which are recruited. The data were collected through a self-administered questionnaire sheet and a physical assessment sheet. **Results**: The study revealed that 94.6% of the girls were circumcised, and 98.2% had their menarche. Overall, 65.5% had satisfactory knowledge, and 81.5% had positive attitude. The main sources of information were classroom whereas parents, newspapers, and magazines were less reported. There was a statistical significance difference between knowledge and had circumcision (p=0.002), also, between knowledge about reproduction and their related attitudes and beliefs (p<0.001). **Conclusion**: unmet information needs were related to female genital mutilation, family planning, pregnancy and labor, and the preferred sources are school book, media, and parents. **Recommendations:** It is recommended that the unmet needs identified should be used for development of educational programs for adolescents. The role of the parents, as well as health care providers needs to be fostered through using of multimedia as television and radio.
[Ragaa A. Hassanain, Sahar N. Mohamed, Nadia H. Ahmed and Mohamed S. Abdel Rahim.**Assessment of female adolescent Reproductive health needs in Assiut city**. Journal of American Science 2011;7(8):103-115].(ISSN: 1545-1003). http://www.americanscience.org.

Key words: Adolescents, reproductive health, knowledge, attitude. Full Text

12 **Ameliorative Effect of Aqueous Leave Extract of *Ocimum Basilicum* on Ccl_4 - Induced Hepatotoxicity and Apoptosis in Albino Rats**

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Abstract: Chemical-induced liver injury depends mostly on the oxidative stress. Basil or sweet basil (*Ocimum basilicum*) is known to have numerous pharmacological activities. The present study **aims** to investigate the effect of basil on Ccl_4-induced hepatotoxicity and apoptotic in albino rats. The result showed CCl_4 caused impairment of the normal structural organization of the hepatic lobules, congestion and dilatation of blood vessels, cytoplasmic vacuolization of the hepatocytes, leucocytic infiltrations and fatty degeneration. The biochemical results showed that there was an increase in serum level of ALT , AST, ALP, cholesterol , triglyceride, , LDL and HDL. Moreover, CCl_4 induced hepatic apoptosis. Treating animals with CCl_4 and aqueous leaves extract of *O. basilica* led to an improvement, in both histopathological and biochemical alterations induced by CCl_4. Also, apoptosis was repaired by shared administration with both *O. basilicum* and CCl_4. These results proved that *O. basilica* had an ameliorative effect against liver injury produced by **CCl_4** due to its antioxidant activity.
[Saber A. Sakr, Sabah F. El-Abd, Mohamed Osman, Asmaa M. Kandil, Mona S. Helmy **Ameliorative Effect of Aqueous Leave Extract of *Ocimum Basilicum* on Ccl_4 - Induced Hepatotoxicity and Apoptosis in Albino Rats**. Journal of American Science 2011; 7(8): 116-127]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: CCl_4 – Hepatotoxicity- rat- *O. basilicum* - Apoptosis Full Text

13 The enhancement anti inflammatory effect of Caffeine on Green tea extract and EGCG on obese rats

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Abstract: Obesity is linked directly with the increased acute phase protein like c-reactive protein (CRP)and increased production of some cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) as a result of an inflammatory signaling pathway. The health benefits of green tea are related to its catechins particularly epigallocatechin-3-gallatel (EGCG) .Green tea contains caffeine which stimulate thermogenesis and fat oxidation. The aim of this study was to evaluate the effect of caffeine on EGCG –and green tea extract–administered obese rats induced by high-fat diet. The animals were divided into two groups. The first group served as healthy control group. The second group was fed high fat –diet for 8 week and was divided into 6 subgroups. IL-6, CRP and TNF-α showed a high significant (p<0.01) increase at zero level and after 5 and 9 weeks of obesity induction as compared to control group. The results showed a decrease in proinflammatory cytokines effect of EGCG, green tea extract and caffeine on TNF-α after 5 weeks (P<0.051,P<0.039,P0.2), for IL6were(P<0.001,P<0.001,P<0.000) , and for CRP(P<0.066,P<0.007, P<0.002) respectively The 9 weeks treatment by EGCG, Green tea extract and caffeine on TNF-α (P<0.312, P<0122, P<0.057) ,on IL-6 (P<0.155,P<0.029,P<0.033), on CRP (P<0.387 P<0.168, P<0.088). The addition of caffeine to green tea showed enhancement of the effect with decrease of TNF-α , IL-6 and CRP after 5 weeks (P<0.03, P<0.001 P<0.034) respectively where after the 9 weeks all the parameters reversed back to normal .The addition of caffeine to EGCG after 5 weeks showed enhancement of the effect on TNF-α, IL-6 and CRP |(P<0.04, P<0.011, P<0.066) respectively, at the end of the 9 weeks all the inflammatory parameters return to normal levels. As conclusion, the addition of caffeine to EGCG or green tea extract enhance their anti-inflammatory effects on the low grade inflammation state a combined obesity by decreasing the levels of TNF-α,IL-6 and CRP.
[Amany SE. Elwakkad,Diaa. B Elazhary ,Saadia A.Mohamed, Salwa R.Elzayat, Mohamed Abd allah**. The enhancement anti inflammatory effect of Caffeine on**

Green tea extract and EGCG on obese rats. Journal of American Science 2011; 7(8): 128-138]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: green tea, EGCG, Caffeine Full Text

14 **Effects of Prenatal Phenytoin Toxicity on the Expression of Glial Fibrillary Acidic Protein (GFAP) in the Developing Rat Cerebellum**

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Abstract The Cerebellum is a highly organized structure and its postnatal development was characterized by cell proliferation, migration and differentiation. Phenytoin is a primary antiepileptic drug used for all types of epilepsy except absence seizures. Even with the newer antiepileptic drugs, phenytoin continues to serve as a reference point and many epileptic women received phenytoin during pregnancy. The mechanism of teratogenicity by phenytoin is still under investigation. So, the present study was designed to clarify the effect of prenatal phenytoin on the cerebellar development in rat offspring using the immunohistochemical distribution of glial marker. The pregnant rats were received phenytoin 35mg/ kg body weight once a day from gestational days E5 to E20. In H and E stained sections, the Purkinje cells in the treated group (PD7) had poor and immature arbors and partially showed an irregular arrangement. There is dispersal of the internal granular cell layer and the white matter with the presence of vacuolations, dilated capillaries and extracellular oedema. The marker of radial glia, glial fibrillary acidic protein (GFAP) has been used to describe phenytoin induced alteration in the morphology and reactivity of Bergman glial cells and their fibres that are the guide substrate of granule cells. The feature of these fibres gives information on the proper granule cell migration. GFAP positive immunoreactivity was first detected at postnatal day one (PD1). Thin glial positive fibers had a regular feature running in parallel in the molecular layer and in the external granular layer of controls at postnatal day seven (PD7). In contrast, in the treated rats, the glial fibers appeared twisted, thickened with an uneven course and strongly labeled end feet. GFAP immunoreactivity in the white matter astrocytes was highly detected in both the control and the treated PD7. From the previous findings, it could be concluded that phenytoin has degenerative changes on the cerebellar development. These changes can lead to extensive neurological poor health effects later in life.
[Sahar Youssef; Olfat A. Abd-El- Aty; Halla H. M. Mossalam and Amina M. A. Tolba. **Effects of Prenatal Phenytoin Toxicity on the Expression of Glial Fibrillary Acidic Protein (GFAP) in the Developing Rat Cerebellum.** Journal of American Science 2011;7(8) 139-152]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Cerebellum; Phenytoin; Purkinje cells; Bergman glial fibers; Astrocytes; GFAP Full Text

15 **Stability Measurement Of Immediate Dental Implants During Healing Process Using Resonance Frequency Analysis**

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Abstract: Primary implant stability has been identified as a prerequisite to achieve osseointegration. Recently, Resonance Frequency Analysis (RFA) has been introduced to provide an objective measurement of implant primary stability and implant stability over the healing period. It was hypothesized that determination of a primary stability threshold, provided in terms of a defined Implant Stability Quotient (ISQ) value, might be relevant to predict the osseointegration of a given implant. The purpose of the current study was directed to evaluate the Osstell ™mentor (Integration Diagnostics AB, Gothenburg, Sweden) as a diagnostic tool capable of discriminating between stable and mobile implants and to evaluate cut-off threshold ISQ value at implant placement that might be a predictive of osseointegration and up to 6 months post placement. Moreover, a correlation between ISQ values and the implants-mesial and distal bone density was carried out. Ten patients (8 men and 2 women) required extraction of maxillary anterior or premolar teeth and planned for immediate dental implants were accepted. A total of 12 Implant Direct's Screw Plant ™ implants (www.implantdirect.com) were placed immediately after extraction of teeth in the selected patients (one implant for each patient and only one patient received three implants). All implants were placed using a non-submerged technique.Immediately after placement of the implant, the Osstell ™ mentor was used for direct measurement of implant stability. Then RFA measurements were recorded at one, two, four and six months postoperative. Periapical digital radiographs were taken postoperatively at the predetermined time intervals. The ISQ values over time intervals, as well as, bone density in the mesial and distal sides of the implants were presented as means and standard deviation (SD) values. Paired t-test was used to study the changes by time. Pearson's correlation coefficient was used to determine significant correlations between ISQ values and the implants bone density %. The means ± SD of the ISQ at implant placement was 52.2 ± 5.2. The means of ISQ values at 1, 2, and 4 months after implants insertion were 58.3, 66.3, and 75.2 respectively, and at 6 months was 86.7. There was a statistically significant increase in the mean ISQ values through all periods ($P<0.001$). The lowest ISQ obtained at implant placement that might be predictive of osseointegration was 49. The results showed a positive correlation between ISQ values and mesial and distal bone density percentage. In conclusion, RFA with the Osstell monitor has been claimed to be useful for monitoring implant stability and osseointegration during the healing phase. The RFA method, as a diagnostic tool, was reliable in identifying implant stability and successful osseointegration for implants with an ISQ ≥ 49. The ISQ values increased gradually with time in correlation with the increased mesial and distal bone density percentage.
[Gamal M. Moutamed. **Stability Measurement Of Immediate Dental Implants During Healing Process Using Resonance Frequency Analysis.** Journal of American Science 2011;7(8) 153-164](ISSN: 1545-1003). http://www.americanscience.org.

***Key words**:* Clinical study, immediate dental implants, ISQ, implant primary stability, Resonance-frequency analysis Full Text

16 **Photodegradation of o-cresol by ZnO under UV irradiation**

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Abstract: The effective removal of o-cresol is currently both an environmental problem. This paper shows how degradation of o-cresol was carried out, in the presence of UV; by ZnO as photocatalyst. To measure the efficiency of photodegradation, the different variables studied included amount of photocatalyst, concentration of o-cresol and pH. The results showed photodegradation was favorable in the pH 6-9 range with amount of photocatalyst 2.5g/L, and o-cresol concentration 100ppm. The detected intermediates were 2-methylresorcinol, 2.5-hydroxybenzaldehyde and salicylaldehyde. TOC studies show that 94% of total organic carbon is removed from solution during irradiation time. This study indicates the great potential of ZnO to remove aqueous *o*-cresol under UV irradiation.
[Yadollah Abdollahi, Abdul Halim Abdullah, Zulkarnain Zainal, Nor Azah Yusof. **Photodegradation of o-cresol by ZnO under UV irradiation.** Journal of American Science 2011;7(8):165-170]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Photodegradation, *o*-cresol, Photoexcited, ZnO, Photocatalyst, UV-Irradiation Full Text

17 **The Cooperation Acid Rains Effect on to Historical Monuments, a Case Study from Canakkale Province; West Anatolia**

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Abstract: Canakkale Monuments Observing data presented that the acid rains effect to in which has main $CaC0_3$ structure Apollo Smithion, Parion and Alexandria Troas according to particular by DTA graphics. The main reason of standing up of historical monuments in the region is caused by resistant greywacke structure, existence of quartz as a thermodynamically decisive form of SiO_2 has increased the resistance to time and acid rain in the region. Getting originated from industry and transportation which give rise to SO_x and NO_x inputs into the atmosphere on to Canakkale's historical monuments. Water loss coming out or high Uv energy which depends on temperature rise. Preserving measures maximum wet sediments in the seasons of autumn and winter rains effect coming from Balkans to study area, as a result to the density reach to the maximum level.
[Rüştü ILGAR The Cooperation Acid Rains Effect on to Historical Monuments, a Case Study from Canakkale Province; West Anatolia, Journal of American Science 2011;7(8):171-178]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Acid rain, historical monument, environment, Canakkale, Turkey Full Text

18 **Sustainable Agriculture in Malaysia: Implication for Extension Workers**

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Abstract: A global survey revealed that at present sustainable agriculture is implemented on only 3 percent of the total farming land in Asia, Africa, and Latin America. Researchers reported some obstacle to adoption of sustainable agricultural practices such as information on sustainable practices, economic factors, education and information, resistance to change, barriers related to sustainable agriculture technologies, social context, financial and material infrastructure and land tenure constraints. One of the main barriers to adoption of sustainable agriculture reported by farm producers and extension workers is lack of available information. Hence agricultural extension workers as information provider play a vital role for achievement of sustainable agriculture. The challenge extension workers faces are that of promoting sustainable agriculture to encourage farm producers adopt the program. The success of sustainable agricultural program depends on training and education of farm producers. The purpose of this paper is to explore whether extension workers have been sufficiently oriented themselves to their responsibility on sustainable agriculture or not.
[Neda Tiraieyari and Jegak Uli. **Sustainable Agriculture in Malaysia: Implication for Extension Workers**. Journal of American Science 2011;7(8):179-182]. (ISSN:1545-1003). http://www.americanscience.org.

Keywords: Agricultural extension. sustainable agriculture. extension workers. Full Text

19 **Ginger Administration has a Protective Effect on the Liver of Albino Rats Treated with 6-Mercaptopurine Drug**

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Abstract: The leukemia is considered to be one of the most frequent tumors in childhood. The first line of its treatment is 6-mercaptopurine (6-MP). There are many recorded cytotoxic effects for 6-mercaptopurine on tissues such as liver, pancreas and bone marrow. Nowadays the needs for administration of cytotoxic drugs are increased as the incidence of malignancies increases. So we have to search for a safe method that can minimize the side effects of these cytotoxic drugs. The ginger has many benefits as anti-inflammatory and anti-oxidant substance. Some indicated as a protective effect for ginger on the radiotherapy treated rats and also a protection from the hepatotoxic and gonado-toxic drugs were recorded. This work amid to investigate the protective effects of ginger on liver of rats treated by cytotoxic drug (6-mercaptopurine), a drug of choice in maintenance therapy for leukemia in adult and children. Our hope is to minimize the side effects of the cytotoxic drugs with simple and available procedure. ***Material*** The current study had been carried on 40 white albino rats that divided into four equal groups. Group I: was served as control group. Group II: was given ginger extract only. Group III: was given 6- MP. Group IV: was given 6-MP + ginger extract. The following parameters were examined in all groups: 1- The serum Alanine aminotransferase (ALT), and aspirate aminotransferase (AST). 2- The cell morphology and histo-pathological changes in the liver. ***The results*** of the present study showed that treatment with 6-MP alone caused marked signs of cellular degeneration and necrosis of the hepatic tissues, significant increase in the serum levels of ALT ($P<0.001$) and AST ($P<0.001$) enzymes when compared with control group. However, the treatment by ginger along with 6-MP showed marked regeneration and improvement in the hepatic tissues and significant decrease in the serum levels of liver enzymes; ALT ($P<0.001$) and AST ($P<0.001$) when compared with 6-MP treated group. ***Conclusion:*** These findings indicated that ginger has protective effect against 6-MP induced hepatic toxicity.
[Olfat A. Abd-El Aty and Enas N. Morgan. **Ginger Administration Has a Protective Effect on The Liver Of Albino Rats Treated with 6-Mercaptopurine Drug.** Journal of American Science 2011;7 (8):183-190]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Ginger, 6-MP, hepatic toxicity. Full Text

20 **Suitability of Groundwater Quality for Irrigation: A Case Study on Hand Dug Wells in Hantebet Catchment, Tigray, Northern Ethiopia**

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Abstract: The study was conducted in Hantebet catchment area which has a total area of 24.5 km^2. The major objective of the study was to assess the suitability of the groundwater quality for irrigation purpose through hydrochemical investigation of the different hand dug wells in the watershed. Having classified the hand dug wells, using the stratified and random sampling techniques, a total of 20 water samples were selected and collected. Water samples were analyzed for alkalinity, sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+}), chloride (Cl^-), bicarbonate (HCO_3^-), carbonate (CO_3^{2-}), sulphate (SO_4^{2-}-S), and nitrate (NO_3^--N) in Water Works Design and Supervision Enterprise Laboratory Service, Addis Ababa. pH and electrical conductivity (EC) were measured in situ. Sodium Adsorption Ratio (SAR) was computed using sodium (Na^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) concentrations in meq/lit. Chemical data of groundwater samples as plotted in trilinear diagram indicated eight water types, Ca-HCO3, Ca-Na-HCO3, Ca-Mg-Na-HCO3, Ca-Na-Mg-HCO3, Na-Ca-HCO3-SO4, Ca-Mg-HCO3-SO4, Ca-Na-Mg-HCO3-SO4 and Ca-Na-HCO3-SO4. Most of the water samples plotted in the Wilcox plot fall in the zone designated C2-S1 and C3-S1 indicating that the groundwater samples generally have low sodium absorption ratio (SAR) and medium to high salinity hazard associated with them. In terms of salinity, eight samples were excellent for irrigation use and twelve samples were with slight to moderate degree of restriction on the basis of ECw. In terms of infiltration, on the basis of ECw and SAR value, eleven samples pose slight to moderate degree of restriction on irrigation. However, nine samples pose none degree of restrictions to its use for irrigation due to its effect on soil infiltration rates. Groundwater in the study area is suitable for surface and sprinkler irrigation use with no chloride toxicity, and with respect to sodium toxicity. Only one sample shows SAR values above 3 (3.095).
[Abraham Bairu Gebrehiwot, Nata Tadesse, K. Bheemalingeswara, Mokennen Haileselassie. **Suitability of Groundwater Quality for Irrigation: A Case Study on Hand Dug Wells in Hantebet Catchment, Tigray, Northern Ethiopia.** Journal of American Science 2011; 7(8):191-199]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Groundwater, Salinity, SAR, Toxicity, Water quality, Hantebet Full Text

21 **Direct shoot regeneration from leaf, root and stem internode segments of male poplar trees and the molecular analysis of variant regenerated plants**

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Abstract: A regeneration protocol has been developed by using thidiazuron (TDZ) with a high frequency of *in vitro* leaf, root and stem internode induction in male (*Populus alba, Populus tremula* L. and *Populus tremula* L. x *Populus. tremuloides* "Michx"). There were differences in average number of shoots among the different sources of explants, the stem internode explants regenerated shoots more effectively than leaf and root explants. In contrast to root and stem internode explants, leaves had poor regeneration abilities in the case of *P. tremula* and *P. tremula* x *P. tremuloides* "Michx". The highest frequency of adventitious shoot formation was (8.2, 39.2 and 38.3 shoots /explant) for *Populus alba, Populus tremula* L. and *Populus tremula* L. x *Populus tremuloides* "Michx" respectively on a medium containing 0.02 µM TDZ when stem internode explants were cultured. Higher TDZ concentrations significantly stimulated further elongation in the newly formed shoots on the three *Populus* species. *In vitro* regenerated plants were genetically analyzed using RAPD fingerprints. The presence of specific loci in the regenerated plants indicated that no genetic variation existed in the regenerated plants.
[Fadia El Sherif and Salah Khattab. **Direct shoot regeneration from leaf, root and stem internode segments of male poplar trees and the molecular analysis of variant regenerated plants**. Journal of American Science 2011;7(8):200-206]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: *In vitro* regeneration, *Populus alba*, *Populus tremula, Populus tremula* x *Populus tremuloides* "Michx", male trees, TDZ, RAPD. Full Text

22 **Assessment of female adolescent Reproductive health needs in Assiut city**

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Abstract: Adolescents around 17 million in Egypt (2005) have different needs and require different counseling approaches and more information. Adolescence is a critical stage for risk-taking because adolescents are moving toward independence and tend to experiment and test limits**. Aim of this study**: was to determine the problems of female adolescents, and provide information about reproductive health. **Subject and methods**: Cross-sectional analytic study was conducted in nursing secondary schools and technical institutes in Assiut city in Upper Egypt with total number of 514 adolescent female students which are recruited. The data were collected through a self-administered questionnaire sheet and a physical assessment sheet. **Results**: The study revealed that 94.6% of the girls were circumcised, and 98.2% had their menarche. Overall, 65.5% had satisfactory knowledge, and 81.5% had positive attitude. The main sources of information were classroom whereas parents, newspapers, and magazines were less reported. There was a statistical significance difference between knowledge and had circumcision ($p=0.002$), also, between knowledge about reproduction and their related attitudes and beliefs ($p<0.001$). **Conclusion**: unmet information needs were related to female genital mutilation, family planning, pregnancy and labor, and the preferred sources are school book, media, and parents. **Recommendations:** It is recommended that the unmet needs identified should be used for development of educational programs for adolescents. The role of the parents, as well as health care providers needs to be fostered through using of multimedia as television and radio.
[Ragaa A. Hassanain, Sahar N. Mohamed, Nadia H. Ahmed and Mohamed S. Abdel Rahim.**Assessment of female adolescent Reproductive health needs in Assiut city**. Journal of American Science 2011; 7(8): 207-220].(ISSN: 1545-1003). http://www.americanscience.org.

Key words: Adolescents, reproductive health, knowledge, attitude. Full Text

23 **Geoelectrical investigation of external corrosion of earth buried pipeline in the coastal area of Gulf of Guinea**

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Abstract: Earth buried transmission pipeline which is coated and protected by impressed current cathodic protection system have been noted to show evidence of external corrosion in its segment buried within low resistivity geomaterials. The resistivity of the geomaterials within the pipeline environments was investigated using electric drilling technique set-up in Schlumberger array. The earth resistivity measured at Ikot Abasi showed low resistivities (12.41-520Ωm), while the geomaterials at Ikot Osuteng produced high resistivity values (1616-15272Ωm). Potential profiling employing close interval potential survey was used to determine extent of the external corrosion as well as the effectiveness of the cathodic protection system. The potential at Ikot Abasi ranges between 331-910mV while that of Ikot Osuteng ranges between

1117-1811mV. The standard practice protective criteria (SP0169) of -850mV showed that the pipeline segment at Ikot Abasi is under severe corrosion while the segment at Ikot Osuteng is well protected by the cathodic protection system.
[Okiwelu, A. A., Evans, U. F., and Obianwu, V. I. **Geoelectrical investigation of external corrosion of earthburied pipeline in the coastal area of Gulf of Guinea.** Journal of American Science 2011;7(8):221-226] (ISSN: 1545-1003). http://www.americanscience.org.

Key words: Potential, Protection, Cathodic, Resistivity, Corrosion, Pipeline Full Text

24 **Economic Resource Situation of Future Researching (Income gained by females) in Middle East in 1404 (A.H.) Horizon**

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Abstract: From the beginning of 3rd millennium gender indices of strength could be expressive of capability of countries difference in the scale of development. Gender development of strength indices not only pays to rate of political and economical partnership of females, but also embraces the strength of economic resources which is measured by gained income by females and males. Structural change of labor force and entrancing females in various fields of labor and activity because of arising level of females education causes decreasing of gap of their partnership with male cause change of attitude relative to difference of level of incomes and wages between females and male. Achievable observations indicate that there is sexual income gap in all countries and can be very different and even in some cases conversely. However, for example in Brazil, females under 25 years have more average hour wage to their male partners. Since females mostly are working in occupations with low wages, naturally have lower income to the males. Average per capita income by share of wages and non-agriculture incomes for Net national production is lower than mails. But what separates developed and developing countries in the field of income between male and female is the rate of this gap.
[Omolbani Poulab. **Economic Resource Situation of Future Researching (Income gained by females) in Middle East in 1404 (A.H.) Horizon** .Journal of American Science 2011;7(8):227-232] (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Economic Resource, females, developing Countries Full Text

25 **Mathematical analysis of Solutions of Drug Models**

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Abstract: In this paper the behavior of solutions of permanent drug resistance model is discussed.The equilibrium points of two drugs resistance are computed. The local stability near equilibrium pionts is discussed. The boundedness, existance of periodic orbits, global stability of permanent drug resistance are studied. the probability generating function for two drugs resistance model in all possible cases is discussed. The obtained results improve and generalize some known results in the literature.
[M. M. A. El-Sheikh, S. A. A. El-Marouf, and E.M. Khalil. **Mathematical analysis of Solutions of Drug Models.** Journal of American Science 2011;7(8):233-239].
(ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Drug resistance; Local Stability; global stability; Periodic orbits; Probability generating functions. Full Text

26 Body Mass Index as an Assessment Tool for Overweight and Obesity in School Children in El-Qalubia Governorate

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Abstract: Aim of the study was to design BMI (Body Mass Index) charts to the studied group, to determine the prevalence of overweight and obesity in the group and to provide suitable recommendations for prevention overweight and obesity. Methods: This was a cross sectional study that included 500 students collected from primary and preparatory schools, aged 7-15 years and living in El-Qalubia governorate through the academic year 2010/2011. A self administered questionnaire was used; it included some socio-demographic characteristics and measurements for weight and height of students. Data was collected, revised and entered using the statistical package SPSS. Results: Obese students represented 20.4% of students. Obesity increased in younger students with mean age 9.33±2.094 years and increased in male students (55% of all obese students), while overweight increased in female students (54.5% of all overweight students). Female students recorded higher values over males in weight during the first 5 years of age (7-11 years old) then, boys become heavier than girls. Moreover, there was a gradual increase in height of female and male students at age 7-15 years old. In addition, the current study indicated that female students have the higher values of BMI from age 7 to 15 years old than males.
[Ahmed Mahmoud Ezzat, Mahmoud Fawzy EL Gendy, Doaa Refaey Soliman, Ashraf Hassan Mohammed and Hazem Salem Abdel Azeem Abou Ghazy. Body Mass Index as an Assessment Tool for Overweight and Obesity in School Children in El-Qalubia Governorate. Journal of American Science 2011;7(8):240-250]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: BMI (kg/m^2), obesity, and overweight. Full Text

27 **Comparative Histological and Ultrastructural Studies on the Stomach of *Schilbe mystus* and the Intestinal Swelling of *Labeo niloticus***

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Abstract: The present investigation aims to illustrate the histological and ultrastructural differences of the stomach of a carnivorous fish, *Schilbe mystus,* and the intestinal

swelling of a herbivorous fish, *Labeo niloticus*. The stomach of *Schilbe mystus* is morphologically divided into three portions: the cardiac, pyloric and fundic portions. However, the histological examination revealed that the stomach is actually divided into two portions: the cardio-fundic portion and the pyloric one. Moreover, the mucosa of the cardiofundic portion of the stomach of *Schilbe mystus* revealed that it consists of two types of cells: the luminal and glandular cells. The luminal cells, which line the gastric lumen and gastric pits, are tall columnar with apically concentrated secretory granules that have a positive PAS reaction. In addition, the glandular cells, which line the bodies of the gastric glands, are pyramidal in shape and possess scattered secretory granules. The pyloric portion of the stomach has the same structure as the cardio-fundic portion, but the gastric glands are completely absent. The histological examination of the mucosa of the intestinal swelling of *Labeo niloticus* revealed that it is built up of a simple columnar epithelium that contains goblet cells. The ultrastructural examination of the gastric mucosa of the stomach of *Schilbe mystus* revealed three types of cells: the luminal, endocrine and exocrine (oxynticopeptic) cells. The luminal cells are tall columnar and are characterized by the presence of apically aggregated secretory granules and a prominent vesiculotubular system. The gastric endocrine cells are mainly pyramidal in shape and are characterized by numerous secretory granules, which exhibit various sizes and shapes. According to the dominant size of the secretory granules, the endocrine cells are differentiated into three types: type-I, type-2 and type-3 cells. Moreover, the gastric glands of *Schilbe mystus* are made up of a single type of exocrine cells, the oxynticopeptic cells. The oxynticopeptic cells are characterized by the combined features of the mammalian oxyntic and peptic cells. The oxynticopeptic cells have numerous apical microvilli, a well-developed vesiculotubular system, numerous mitochondria, a rough endoplasmic reticulum and numerous secretory granules.The ultrastructural examination of the mucosa of the intestinal swelling of *Labeo ni/oticus* revealed that the enterocytes are tall columnar and possess welldeveloped microvilli, numerous mitochondria, microtubules, a few endocytotic channels and aggregated chylomicrons.

[S. A. A. Naguib; H. A. EI-Shabaka and F. Ashour. **Comparative Histological and Ultrastructural Studies on the Stomach of *Schilbe mystus* and the Intestinal Swelling of *Labeo niloticus*.** Journal of American Science 2011;7(8):251-263]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Stomach histology, Stomach ultrastructure, *Labeo nilotieus, Schilbe mystus,* Teleost. Full Text

28 **Evaluation of Monosodium Glutamate Induced Neurotoxicity and Nephrotoxicity in Adult Male Albino Rats**

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Abstract: Monosodium glutamate (MSG) is a food additive with a wide use in modern nutrition. The Current research concerned with studying the toxic effects of MSG on rats cerebrum and kidneys. Thirty six adult male albino rats were divided into three groups (each containing 12 rats); negative control group, positive control group (received 2 ml saline orally for 28 days) and MSG group (received 830 mg/ Kg. B. wt orally for the same period). Serum creatinine and blood urea nitrogen, urine analysis for urinary albumin excretion and histopathological examination for cerebrum, kidneys were examined in all groups. The results showed marked increase in the serum creatinine, BUN levels in MSG group as compared to control group. These changes were accompanied with a significant increase in the urinary excretion of albumin. These alterations were accompanied by variable histopathological changes of the examined cerebral and renal tissues. There were neurogenerative changes in the form of vacuolization, pyknosis, satellitosis and chroidal plexus congestion in the cerebral cortex. The renal tissue showed swelling in the lining endothelium of the glomeruli associated focal areas of glomerular atrophy. There was also hydropic degeneration of the tubules with tubular dilatation and hyaline casts. The inter-tubular spaces showed dilatation and congestion of the cortical blood vessels with focal hemorrhage between the tubules. Moreover MSG treatment induced up regulation of Bax protein in all examined tissues as compared to control rats indicating that MSG induced apoptosis. In conclusion, the results confirmed the neurotoxic and nephrotoxic effects of MSG, where Bax protein appeared to have a pivotal role in MSG induced apoptosis in these organs.

[Marwa A. Abass and Manal. R. Abd El-Haleem Evaluation of Monosodium Glutamate Induced Neurotoxicity and Nephrotoxicity in Adult Male Albino Rats. Journal of American Science 2011;7(8):264-276]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Monosodium Glutamate; Food Additive; Cerebrum; Kidneys; Apoptosis and Bax immunostaining Full Text

29 **Technology of earthquake resistant solar systems used in solar earthquake parks**

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Abstract: Earthquake is among the natural destructive catastrophes whose incidence imposes serious social and economical damages to human societies. The only way to save people is to pay attention to safety issues, providing the required facilities and equipments prior to earthquake occurrence. The first step is hence smoothing the way for establishing and equipping parks and places supplying the essential needs of people wherein the natural, permanent, and reusable energy is used for the purpose of providing light, air conditioning, cooking, and telecommunication requirements. Regarding such an approach, carrying out the project of solar park for earthquake in all regions of Iran with high probability of earthquake occurrence is of critical importance. The next step will be to use the technology of anti-earthquake solar systems in these parks, which paves the way for reaching the first objective. In order to supply the required energy for light, air conditioning, and also for preparing hot water for bath and washing in these earthquake solar parks, it is needed to install photovoltaic (PV) panels with essential equipments on floor or under the ceiling of the buildings which have been recognized to be resistant against earthquake in critical times. Accordingly, making use of the technology of solar systems and designing and making the buildings with maximum use from solar energy are of great significance.

[Akbar Toloeeyan. Akbar Toloeeyan. Journal of American Science 2011;7(8):277-284]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Solar Park; Reproducible energies; Earthquake; Natural catastrophes; solar systems; Photovoltaic Full Text

30 **Comparative Studies on Some Factors Affecting Rooting Ability of Carob Stem Cuttings**

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Abstract: The present study was carried out during two successive seasons of 2007 and 2008 on 20 – years –old on a Carob (*Ceratonia Silique L.*), female tree which performed the best vegetative growth ,yield and pod quality, grown in the experimental orchard station of Horticulture Research Institute at Giza, Egypt .The aim was to find out the effect of some factors (type of cutting, date of cutting preparation and hormone treatment) on vegetative propagation of Carob plants by stem cuttings. To achieve this work, two types of Carob stem cuttings (terminal and sub-terminal) were prepared from one- year- old branches. Monthly, on each collection date, cuttings

were subjected to the hetero auxin (IBA) treatments by dipping the base of them 10 sec. in 3000, 6000 and 9000 ppm IBA solution, with or without wounding and with or without NAA at two concentrations 100 & 200 ppm. Cuttings were divided into two groups; first group was planted under intermittent mist, and the second group under white plastic tunnel. Results showed that rooting ability of carob, root length and dry weight, survival after 2 months and average number of roots increased by increasing concentration of IBA from 3000 to 9000 ppm with wounding and with NAA at 100 and 200 ppm in both April and May in both terminal and sub terminal cuttings, but decreased to the little extent in August and September. While, fluctuated greatly exhibiting very low values to zero during the period from October to March. Terminal cuttings were the best in the rooting ability, root length and survival after 2 months. Sub-terminal cuttings were the best in the average number of roots and dry weight of roots under mist and tunnel. The untreated Carob stem cuttings of two types appeared to be difficult or impossible to root in collection dates and different concentrations of hormone. In conclusion, vegetative propagation of Carob by cuttings under mist or under tunnel affected by many factors such as: time of preparing cuttings, type of cuttings and treated with growth regulators. Moreover, mist propagation technique costly where as in areas characterized by poor facilities, propagation under white plastic tunnels is cheaper and save the cost of electricity and can be used commercially.

[Shereen, A. Shaheen and Aly A. A. **Comparative Studies on Some Factors Affecting Rooting Ability of CarobStem Cuttings**. Journal of American Science 2011;7(8):285-301] (ISSN: 1545-1003). http://www.americanscience.org.

Key words: Carob tree,type of cutting, date of cutting preparation and hormone treatment, stem cutting types Full Text

31 **Sultanistic regimes, an appropriate model for leaving the problems of bilateral explanations of authoritarianism and totalitarianism**

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Abstract: Reviewing the history for the structure of power in the 3rd world expresses the principles of long time existence of non-democratic systems in power. Hence we observe the establishment of Eastern despotic models , Eastern inheriting sultanistic regimes, Asian inheriting despotism and the theories of sultanate systems, expressing this type of structure of power .The theory of sultanistic regimes is a rather modern model , with numerous and different characteristics , than other views , benefiting more strong points and less errors. Therefore , this article is dealing with considering this theory.

[Shiva Jalalpour. Sultanistic regimes, an appropriate model for leaving the problems of bilateral explanations of authoritarianism and totalitarianism. Journal of American Science 2011;7(8):302-307] (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: sultanistic regimes, authoritarianism, totalitarianism Full Text

32 **Synthesis and Biological evaluation of pyrrolo[2, 3-*d*]pyrimidine derivatives as antibacterial and antiviral**

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Abstract: A new class of pyrrolo[2,3-*d*] pyrimidine derivatives has been designed and synthesized, then examined and evaluated for their antiviral and antibacterial activity. New prophylactic therapeutic tools are needed for the treatment of highly pathogenic avian influenza (HPAI) H5N1 and highly virulent Newcastle disease virus (NDV). **3a, 3b, 3g, 3h** have shown to posses highly potent against highly pathogenic avian influenza (HPAI) H5N1 virus and **1a, 2a** are shown highly potent against highly virulent Newcastle disease virus (NDV), and the compounds **1a-c , 2a, 2b, 3a, 3g, 3h** are shown highly potent against enterobacteriases [*Escherichia coli* and *Salmonella typhimurium*] strains. This study is the first record in Egypt and may be in the world concerning the activity of these new class of pyrrolo[2,3-*d*]pyrimidine derivatives against (HPAI) H5N1 and NDV.

[Khaled M. Hassan Hilmy, Mounir D. Elsafty, Alaa Ramadan. I. Morsy, Ghada M. Elsadek Aly and Salah M. El-kousy. Synthesis and Biological evaluation of pyrrolo[2, 3-*d*]pyrimidine derivatives as antibacterial and antiviral. Journal of American Science 2011; 7(8):308-314].(ISSN: 1545-1003). http://www.americanscience.org.

Key words: Pyrrolo[2, 3-*d*]pyrimidine derivatives , antibacterial , antiviral activity Full Text

33 **Perceived Self-efficacy and Commitment to an Exercise Plan in Patients with Osteoporosis and Osteoarthritis**

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Abstract: Exercise remains a central health behavior in improving osteoporosis and osteoarthritis. A convenience sample of patients with a clinical diagnosis of osteoporosis (75) and osteoarthritis (75) was recruited. Their mean age ±SD was 52.9±7.2 and 50.0±13.1, respectively. Obtained results revealed that osteoporotic patients had significantly higher mean scores of commitment to plan, and higher exercise self-efficacy, while the mean score of exercise benefit was higher in the osteoarthritis group. In addition; a significant positive correlations were found between commitment and perceived self-efficacy scores, and between self-efficacy and perceived lack of barriers and exercise benefits in both groups. In regression analysis, age was a negative predictor for commitment in osteoporosis group, while self-efficacy and lack of barriers were positive predictors. In osteoarthritis group, self-efficacy was the only positive predictor of commitment. Based on these results, it is recommended that the heath care professionals should adopt strategies for enhancing patient's self-efficacy, give special attention to perceived barriers to exercise, and tailor exercise interventions to the different needs and perceptions of the patients with osteoporosis and osteoarthritis. The aim of this study was to compare perceived self-efficacy, exercise benefits, exercise barriers, and commitment to exercise between patients diagnosed with osteoporosis and osteoarthritis, and assessing the influence of perceived exercise self-efficacy, exercise benefits, and barriers on commitment.

[Hanan, S. A. and Sahar, Y. M., Perceived Self-efficacy and Commitment to an Exercise Plan in Patients with Osteoporosis and Osteoarthritis. Journal of American Science 2011;7(8):315-323].(ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Osteoporosis; Osteoarthritis, Exercise, Self-efficacy, Commitment Full Text

34 **The impact of premenstrual syndrome on social Participation of the 25-35 year old female staffs of Baft city (Kerman Province) in 2010**

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Abstract : Development is not materialized without the women's contribution, so that preparing an appropriate ground for different activities of the women and fighting against all barriers of women participation in socio-economic and political activities is essential. One of the barriers is the existence of the Premenstrual Syndrome (PMS) in women. PMS is a set of disturbing signs which are experienced by many people some days before menstruation. Many of pro-women thinkers believe that PMS is a psychological disturbance being created by medical science to treat a natural biologic process, so that to prevent of the women interference in heavy and strong activities in the society. In order to determine the existence of PMS and its consequences, a study was done on 130 women in 25 to 35 years old who were the female employed staff in the public offices in Bam city. It was a survey and in order to collect data, in addition to questionnaires, books and other documents were also used. The results show that the percentage of affliction with PMS was 94%. The affliction with depression resulting from the syndrome was reported in 70% of the individuals and those afflicted with agony were 64%. This high pain of PMS affliction along with the consequences resulting from the physical signs, distress and depression can have a threatening factor for the women's health and a pretext to make employers disappointed of employing women. Journal of American Science 2011; 7(8):324-328]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: Women, Premenstrual Syndrome, Distress, Depression, Social Participation Full Text

35 **Experimental Equality and Under Determination**

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Abstract: During this century, there emerged from the philosophical analysis of scientific theories two results invested with broad epistemological significance By the 1920s, it was widely supposed that a perfectly general proof was available for the thesis that there are always empirically equivalent rivals to any successful theory. Secondly, by the 1940s and 1950s, it was thought that - in large part because of empirical equivalence - theory choice was radically underdetermined by any conceivable evidence. Whole theories of knowledge (e.g. , W. V. Quine's) (Fraassen. 1976) have been constructed on the presumption that these results were sound; at the same time, fashionable recent repudiations of the epistemic project (e.g., Richard Rorty's) have been based on the assumption that these results are not only legitimate, but laden with broad implications for the theory of knowledge. In this paper, we reject both the supposition of empirical equivalence and the inference from it to underdetermination. Not only is there no general guarantee of the possibility of empiricalk equivalent rivals to a given theory, but empirical equivalence itself is a problematic notion without safe application. Moreover, the empirical equivalence of a group of rival theories, should it obtain, would not by itself establish that they are underdetermined by the evidence. One of a number of empiricalk equivalent theories may be uniquely preferable on evidentially probative grounds. Having, argued for these conclusions in the first two sections, respectively, we shall propose, in section III of this paper, a diagnosis of the difficult that has impeded their recognition, and extract in attendant, positive moral for the prospects of epistemology.
[Ramin Seyedi, Experimental Equality and Under Determination. Journal of American Science 2011;7(8):329-337]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Wavelet Transform, Harmonic Analyses, Signal and Damage Full Text

36 **The evaluation of different levels of Manta pulagum on performance, and blood parameters of broilers**

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Abstract: This study was conducted to investigate the effects of using different levels of Menta pulagum on performance, carcass traits, blood biochemical and immunity parameters of broilers. At first 300 one day old broiler chicks were divided to 5 groups and five replicates of 12 chicks in each group. Experimental groups included T1, control group with no Menta pulagum supplementation, T2, T3, T4, and T5 received 0.75%, 1%, 1.5%, and 2% Menta pulagum respectively. The results showed that the use of different levels of Menta pulagum has significant effects on performance and carcass traits of broilers ($P<0.05$). The highest level of weight gain was in group 5 also the highest percent of liver and breast were observed in group 5 but the greatest percent of thigh was observed in group 4. The results evidence that the using of Menta pulagum in broiler feeds have significantly effects on performance and blood biochemical and carcass traits without having any significantly effects on immunity parameters except the level of heterophile to lymphocyte.
[Navid Hosseini Mansoub. The evaluation of different levels of Menta pulagum on performance, and blood parameters of broilers. Journal of American Science 2011;7(8):338-341]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Blood parameter, Broiler, Performance, Menta pulagum Full Text

37 **Evaluating Competition of the *Phalaric minor* in Wheat**

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Abstract: to competitive effects of wheat and *p minor* in densities and different use values of Nitrogen in plan frame of random blocks repeating 4 times perfectly. The test factorials included Nitrogen value in level (100,150 and 225 kg/ha) and *p minor* density in 5 levels (0, 20, 40, 80 and 160 bones per square meter). Results of the test showed that height of *p minor* per three values of Nitrogen was less in initial processes of growth and more than wheat in final processes of growth. Increasing Nitrogen value has caused to increase leaf and biomass of *p minor* and increasing *p minor* density causes area of leaf and biomass of wheat to decrease. Time of closing canopy in *p*

minor is more rapidly than in wheat. The most value of decreasing operation of wheat seed was about 48 percent while was observed in density of 160 bones of *p minor* in 225 kg/ha of Nitrogen. Average relative growth velocities of wheat and *p minor* were 0.073 and 0.028 g/dag during growth cycle, respectively. Little use value of Nitrogen fertilizer, reason of more decrease of wheat operation was existence of *p minor* grass.
[Einallah Hesammi Faculty member, Department of Agronomy, Islamic Azad University, Shoushtar Branch. Iran. Journal of American Science 2011; 7(8):342-346]. (ISSN: 1545-1003). http://www.americanscience.org

Keywords: Wheat, *p minor*, Competition, Nitrogen, Density Full Text

38 **Heavy Metals Enrichement in Deposited Particulate Matter at Abu Zaabal Industrial Area –Egypt**

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Abstract: This study aims to assessment the impact of two major polluted industries in Egypt located in Abu Zaabal industrial area. Deposited particulate matter was monitored at Abu Zaabal industrial area around Awadalla lead smelter and abu Zaabal phosphorus fertilizer plant. This study also provides information on the distribution of trace elements in dust fall during different times and areas. Dust fall was collected monthly from November 2008 to October 2010. Heavy metals were extracted by nitric acid and measured using Perkin Elmer 6100 ICP/MS. Dust fall (DF) results revealed that the annual mean rate of deposited dust collected during the year 2009 was 29.49, 33.54, 35.64, and 36.83 g/m^2.30days for sites no. 1, 2, 3 and 4 respectively while during the year 2010, the annual mean rate of deposited dust was 28.53, 34.40, 164.46 and 295.65 g/m^2.30 days for sites no. 1, 2, 5 and 6, respectively. Sites no 5 and 6 recorded higher P_2O_5 concentration and deposition rate than sites 1 and 2 that is due to the impact of fertilizer plant. High enrichment factors for heavy metals (Pb, Cd, and Ni) were found in dust-fall samples, while Zn, Co and Cr showed minimal to moderate enrichment.
[Alia A. Shakour Ali, Nadia M. El Taieb, Ali M Ali Hassan. Yasser H. Ibrahim and Sabry G. Abd El Waha. Heavy Metals Enrichement in Deposited Particulate Matter at Abu Zaabal Industrial Area –Egypt. Journal of American Science 2011;7(8):347-352]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: Industrial area, Deposited particulate matter, Heavy metals. Full Text

39 **Polyamide 6.6 planed Polyester: A New Prosthetic Fabric for Repair of Superficial Digital Flexor Tendon Deficit in Equine**

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Abstract: A new prosthetic material polyamide 6.6 planed polyester was used for repair of lacerated and cut digital flexor tendons in equine especially those accompanied by deficits and gap formation. This work was done experimentally on 17 donkeys and applied on 5 clinical cases .The prosthetic material was placed and fixed between the two cut ends to reconstruct the tendinious defect. Clinical, ultrasonographic, histopathologic and biomechanical evaluations were performed to judge the efficiency of the prosthetic implant. Satisfactory results were obtained regarding healing of the affected tendons, the return of the animals to their normal ambulation and gait. Clinical cases showed good healing in extensor tendon lacerations and superficial digital flexor cuts. Complications were encountered in a case of deep digital flexor cut with large gap. It was found that, the new Polyamide 6.6\ Polyester fabric (1:1) proved to be strong and biocompatible in addition its low cost in comparison with other prosthetic material.
[Inas N. El-Hussiney, Torad F.A., Ali K.M., Ali Marwa A. and Ibrahim.M.I. Polyamide 6.6\ Polyester: A New Prosthetic Fabric for Repair of Superficial Digital Flexor Tendon Deficit in Equine. Journal of American Science 2011; 7(8):353-361]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: prosthetic material; polyamide; tendon; equine; Polyester; fabric Full Text

40 **Synthesis, Isomerization, and Antimicrobial Evaluation of Some IndenothienoPyrimidine Derivatives**

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Abstract: 9-Hydroindeno[1¢,2¢:4,5]thieno[2,3-d]pyrimidin-4-ylhydrazine**(2)**was used as a precursor for preparation of some novel 2-substituted [1,2,4] triazolo[1,5,-c]-9H-indeno [1¢,2¢:4,5]thieno[3,2-e]pyrimidine derivatives **(5,6,8)**and 9H-indeno[1¢,2¢,:4,5]thieno[3,2-e]tetrazolo [1,5-c] pyrimidine **(10)**. Furthermore, the preparation of N-substituted-9-H-indeno [1¢,2¢: 4,5]thieno[2,3-d] pyrimidin-4-yl) amine derivatives**(11-14)** were described. Also, thieno[2,3-d] pyrimidin-4-yl-isothiourea derivatives **15** was obtained from reaction of **1** with thiourea. Selected members of the prepared compounds were screened for antimicrobial activity.
[Nermin A. Marzouk, Ahmed H. Shamroukh, Abeer H. Al-Saadny, J. A. Micky and Farouk, M. E.Abd El-Megeid Synthesis, Isomerization, and Antimicrobial Evaluation of Some Indenothieno Pyrimidine Derivatives. Journal of American Science 2011; 7(8):362-369]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Antimicrobial evaluation; 4-substituted indenothieno-pyrimidines; rearrangement; thienotriazolopyrimidines; thienotetrazolopyrimidine Full Text

41 **Economic Analysis of Foreign Trade between Egypt and countries of the Great Arab Free Trade Agreement**

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Abstract: Establishment of Great Arab Free Trade Agreement (GAFTA) is considered one of the most important economic achievements among Arab countries, where the declaration of GAFTA is an important factor to accelerate the process of canceling of tariff and non-tariff barriers on trade among Arab countries. The research problem is concerned with the volume of trade between Egypt and GAFTA countries is relatively small, so the object of this research is to try to access how to increase the volume of trade between Egypt and country members in the GAFTA, and to identify the most important variables affecting Egyptian exports and imports with GAFTA, and to identify the most important countries that are expanding or limiting in imports from Egypt. The results showed that Saudi Arab was ranked as the first in the list of

Egyptian exports, which ranged between 21.7%, 20.4% during 2005, 2009. As it turns out that Kuwait was as the ranked first in the list of Egyptian imports in 2005 by 33.6% of the total value of Egyptian imports from these countries. Exports of Saudi Arab to Egypt were in the first rank during the period (2006-2009) by 37.8%, 48.4%, 44.3%, 42.3%, respectively. The results of the gravity model showed that an increase in per capita GDP in each of Egypt and GAFTA countries lead to increase exports and imports, while increasing the geographical distance between Egypt and GAFTA countries lead to a decrease both of them. Gravity model for exports showed that to Sudan and Syria response to the demand for Egyptian exports at high levels of income of individuals, while the gravity model for imports showed the Egypt response to import from Saudi Arabia, UAE, Libya, and Kuwait. The study therefore recommended the need to develop joint infrastructure projects, and improve the means of transport, particularly two states, with neighboring Libya and Sudan, and export of good and services take into account consumer taste and quality requirements.
[Eman Abd-Elghafour Ahmed. Economic Analysis of Foreign Trade between Egypt and countries of the Great Arab Free Trade Agreement. Journal of American Science 2011;7(8):370-376]. (ISSN: 1545-1003). http://www.americanscience.org.

Key Words: Great Arab Free Trade Agreement (GAFTA) Gravity Model Egyptian Exports, Egyptian Imports, open door policy. Full Text

42 **Halophytic Plants for Phytoremediation of Heavy Metals Contaminated Soil**

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Abstract: Using of halophyte species for heavy metal remediation is of particular interest since these plants are naturally present in soils characterized by excess of toxic ions, mainly sodium and chloride. In a pot experiment, three halophyte species *viz*. *Sporobolus virginicus*, *Spartina patens* (monocotyledons) and *Atriplex nammularia* (dicotyledon) were grown under two levels of heavy metals: 0 level and combinations of 25 mg Zn + 25 mg Cu + 25 mg Ni/kg soil. The three species demonstrated high tolerance to heavy metal salts in terms of dry matter production. *Sporobolus virginicus* reduced Zn, Cu, and Ni from soil to reach a level not significantly different from that of the untreated control soil. Similarly, *Spartina patens* significantly reduced levels of Zn and Cu but not Ni. *Atriplex nummularia* failed to reduced Zn, Cu and Ni during the experimental period (two months). Only *Sporobolus virginicus* succeeded to translocate Zn and Cu from soil to the aerial parts of the plant. The accumulation efficiency of Zn and Cu in aerial parts of *Sporobolus virginicus* was three and two folds higher than *Spartina patens* and around six and three times more than *Atriplex nammularia* for both metals, respectively.
[Eid, M.A. Halophytic Plants for Phytoremediation of Heavy Metals Contaminated Soil. Journal of American Science 2011; 7(8):377-382]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: Spartina *patens, Sporobolus virginicus, Atriplex nummularia*, Zn, Cu and Ni, Phytoremediation Full Text

43 **Effects of Chronic Exposure to Static Electromagnetic Field on Certain Histological Aspects of the Spleen and Some Haematological Parameters in Albino Rats**

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Abstract: Over the past few years, our environment has become seething electromagnetic smog that bombards our bodies every second of every day. Because electro-magnetic fields are invisible, we do not even realize they are there, although they are battering us mercilessly. Special attention has been given to the biological effects of magnetic fields. Thirty six male albino rats *(Rattus norvegicus*) were utilized in the present work to study the effects of static magnetic field (SMF) equaling 2 ml tesla on the spleen and some haematological parameters. Magnetic exposure was applied for 60 minutes for 3 days per week for two weeks. One day following magnetic exposure, the spleen showed congestion in the splenic sinusoids accompanied with thickening of the splenic capsule. A significant increase in the white blood cells and blood platelets was accompanied by enlargement of the white pulp were detected. Seven days following magnetic exposure, an increase of haemoglobin concentration; haematocrit and red blood cells was recorded accompanied with a highly significant decrease in blood iron. Later on, such increase was followed by a significant decrease in most haematological parameters after fifteen days of magnetic exposure. Hemosiderin granules were observed in the dilated splenic sinusoids at the areas of congestion. The splenic tissues and the haematological parameters appeared almost normal and manifested a tendency towards recovery after thirty days following magnetic exposure.
[Mervat S. Zaghloul . **Effects of Chronic Exposure to Static Electromagnetic Field on Certain Histological Aspects of the Spleen and Some Haematological Parameters in Albino Rats**. Journal of American Science 2011;7(8):383-394]. (ISSN: 1545-1003). http://www.americanscience.org.

Keyword: Static Electromagnetic Field Histological Aspects Spleen Haematological Parameters in Albino Rats Full Text

44 **ETFRC: Enhanced TFRC for Media Traffic over Internet**

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Abstract: The evident increase in media traffic over Internet is expected to worsen its congestion state. TCP-friendly rate control protocol TFRC is one of the most promising congestion control techniques developed so far. TFRC has been thoroughly tested in terms of being TCP-friendly, responsive, and fair. Yet, its impact on the visual quality and the peak signal-to- noise ratio PSNR of the media traffic traversing Internet is still questionable. In this paper we aimed to point out the enhancements required for TFRC that enables producing the maximum PSNR value for Internet media traffic. Firstly, we suspected the default value of n that represents the number of loss intervals used in calculating the loss event rate in the TFRC equation. This value is recommended to be set to 8 according to the latest RFC of TFRC. We investigated the effect of modifying the TFRC mechanism on the resulting PSNR of the transmitted video over Internet using TFRC via switching n across the values from 2 to 16. We investigated the effect of such variation over a simulated network environment to study its effect on the resulting PSNR for a number of arbitrary video sequences. Our simulations results showed that running TFRC with n=11 led to reaching the maximum PSNR values among all the examined values of n including its default value. Secondly, we tested the impact on the PSNR of another modification in the TFRC mechanism via switching both values of n and Nfb which is frequency of feedback messages sent by TFRC receiver to its sender every round-trip time RTT. The default value of Nfb is 1; hence we scanned every possible combination of n and Nfb ranging from 2 to 16, and from 1 to 4, respectively and recorded the produced PSNR. It was obvious that several other combinations of n and Nfb produced higher PSNR values

other than their default values in the request for comment RFC of TFRC. We hereby suggest using an enhanced TFRC that we abbreviated as ETFRC which has the values of n and Nfb value set to 4 and 11 respectively as a replacement for the traditional TFRC to enable reaching higher PSNR for media traffic over Internet.
[Mohammad A. Talaat, Magdi A. Koutb, and Hoda S. Sorour. ETFRC: Enhanced TFRC for Media Traffic over Internet. Journal of American Science 2011;7(8):395-402]. (ISSN: 1545-1003).
http://www.americanscience.org.

Keywords: TFRC, Congestion Control, PSNR Full Text

45 **Combination toxicity effects of heavy metals on terrestrial animal (Earthworm-** *Eisenia andre)*

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ABSTRACT: Combination toxicity of heavy metals to earthworms has been studied for the first time. Three metals, namely Cadmium (Cd), Lead (Pb) and Zinc (Zn) were used as toxicants. The study was carried out in line with the OECD recommended procedure for ecotoxicology tests of chemical substances to soil. Hence, the OECD approved earthworm species Eisenia Andrei was used as biological test organism. OECD artificial soil was used as test substrate. The parameters of interest in the study were the effect of logarithmically increasing concentrations of single, and finally of combinations of metals, to reproduction and body growth of earthworms. These parameters were chosen for the study since sublethal effect on growth and reproduction are of more ecological relevance than an acute toxicity effect studying mortality. Growth was measured as the increased in body weight of the worms in time. Reproduction was estimated based on the number of cocoons produced per worm and the number of emerged juveniles produced per cocoon. The EC50 values of the effect of the toxicants to the organisms were estimated by an extended logistic model which is able to take into account the presence or absence of hormesis in calculating EC50 values. The EC50 values after three weeks of the three metals to growth and cocoon production were first determined singly. Cocoon production and juvenile numbers appeared to be more sensitive parameters than growth. A continuous increase in weight was observed in almost all tested concentrations of each metal except at the highest concentration of lead. Cd appeared to have the strongest adverse on cocoon production, followed by Zn, and then Pb. This is judged from the observed EC50 values of each of the metals on cocoon production. The observed EC50 values were 104.0mg/kg (1.076 mmol/kg), 418.3mg/kg (6.398 mmol/kg), 1570.3mg/kg (7.578 mmol/kg) for Cd, Zn and Pb respectively. Based on the observed EC50 values of the single toxicity tests, combination toxicity tests were carried out for Zn & Cd, Cd & Pb and Pb & Zn. Zn & Cd were found to be antagonistic (less than concentration additive) other tests where not interpretable. The ecological implication of the result of combination toxicity test of Cd & Zn is that single toxicity test alone may not indicate the fate of earthworms exposed to sites polluted with combinations of metals.
[Paul Chuks Onuoha, Worgu, D.C. **Combination toxicity effects of heavy metals on terrestrial animal (Earthworm-** *Eisenia andre)*. Journal of American Science 2011;7(8):403-415]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: toxicity; heavy metal; terrestrial animal; Earthworm; *Eisenia andre* Full Text

Bacillus Calmette-Guérin versus Gemcitabine for Intravesical Therapy in Intermediate and High-Risk Superficial Bladder Cancer 46

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Abstract: Bacillus Calmette Guerin (BCG) has been the mainstay of intravesical treatment, however, its clinical effectiveness is accompanied by a wide range of adverse events. Gemcitabine has a good safety profile with promising features for the use against intermediate risk non-muscle invasive bladder cancer (NMIBC). It can be a potential chemotherapeutic drug for high- risk patients. The aim of this study was to evaluate the safety and efficacy of adjuvant intravesical gemcitabine versus BCG in the treatment of intermediate and high -risk NMIBC. Patients and methods: Between May 2006 and April 2008, a total of 57 patients were randomized into 2 groups; group I: 28 patients, were treated with six weekly intravesical instillation of BCG and group II: 29 patients, received six weekly intravesical grmcitabine. Patients were evaluated for response, at 8 weeks, then every 3 months. Outcome measures were response rate, overall recurrence rate, progression rate, median recurrence free period, median progression free period and 1-year recurrence free survival. Treatment related complications were also evaluated. Results: For intermediate risk patients, there was no significant difference between the two groups in the complete response (CR) rate (93.3% vs. 87.5%), the overall recurrence rate (33.3%vs.25%), the progression rate (6.7% vs. 6.2%), and the median progression free period (13 vs. 16 months). However, the median recurrence free period was longer for group I compared to group II (18.5 vs. 15 months) and the difference was statistically significant. Kaplan-Meier curve showed that there was no significant difference between the two groups in the 1-year recurrence free survival (95.3% vs. 98.7%) and the median recurrence free survival (22 vs.18 months). For high risk patients there was no significant difference between the 2groups in CR rate (61.5% vs. 76.9%), the progression rate (15.4% for both groups) the median recurrence free period (15 vs. 14 months) and the median progression free period (17 vs. 15 months). However, the overall recurrence rate of group I was lower than that of group II (7.7% vs. 30.8%) and the difference was statistically significant. Kaplan-Meier curve showed that there was no significant difference between the two groups in the 1-year recurrence free survival (76.9% vs. 69.2%) and the median recurrence free survival (18 vs.15 months). The adverse events of group I were more marked than that of group II. Conclusion: Gemcitabine is active and well tolerated for intravesical instillation.It is considered to be an efficient treatment for intermediate risk NMIBC. However, for high- risk group, it is inferior to BCG, but owing to its favorable toxicity profile, it may be useful for patients intolerant to BCG.
[Ahmed M. Abd-Alrahim and Hoda H. Essa, Bacillus Calmette-Guérin versus Gemcitabine for Intravesical Therapy in Intermediate and High-Risk Superficial Bladder Cancer]. Journal of American Science 2011;7(8):416-426]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Bacillus Calmette-Guérin; Gemcitabine; Intravesical Therapy; Bladder Cancer Full Text

47 **Effect of a Rehabilitation Program on the Knowledge, Physical and Psychosocial Functions of Patients with Burns**

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Abstract: Burns injuries may be severe enough to restrict daily activities in the home, school, and workplace. The nurse has a crucial role in implementing strategies for

relevant effective care of patients with burn. The aim of the study was to evaluate the effect of a rehabilitation program on the knowledge, physical and psychosocial functions of patients with burn. This quasi-experimental study was conducted at the burn units of the Main University and Emergency Hospital in El-Mansoura on a convenience sample of 100 burn patients who were divided randomly into two equal groups: a study group for application of the intervention, and a control group to receive the routine management of the hospital. Data were collected using an interview questionnaire form and the Brief Burn Specific Health Scale (BSHS-B). A rehabilitation program with a booklet was developed after reviewing related literature to cover burn definitions, causes, types of burn, complications, proper diet, exercise, wound care, stress management. A booklet containing all the program materials was prepared in Arabic language. The program was provided to the study group patients, while the control group received the routine hospital's nursing management. The program effectiveness was evaluated through a posttest done for both groups. The study was implemented during the period from November 2006 to November 2007. Patients in the two groups had similar demographic as well as burn injuries characteristics. After implementation of a rehabilitation program, the knowledge scores were statistically significantly higher among study group patients in all areas as burns definition ($p<0.001$), treatment ($p=0.022$), exercise ($p<0.001$), and diet ($p< 0.001$). They also had significantly better scores of the Brief Burn Specific Health Scale (BSHS-B). The scores of knowledge and BSHS-B improved in both groups; however, the magnitude of the change was much lower in the control group compared to those in the study group. The study concludes that inclusion of the information needed by patients with burn in a rehabilitation program improves their knowledge, with consequent positive impact on their physical and psychosocial status. Therefore, the study recommends implementation of this rehabilitation program for all patients with burn injury. Nurses should be trained in patient education in order to be able to successfully implement such program.

[Mohamed Radwan, Salwa Samir, Ola Abdel Aty and Salwa Attia Effect of a Rehabilitation Program on the Knowledge, Physical and Psychosocial Functions of Patients with Burns. Journal of American Science 2011;7(8):427-434]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: Burn, rehabilitation program, nursing Full Text

48 **Detection of Genetic Variation Among Three Isolates of *R. leguminosarum* Using Protein, Isozyme and DNA Fingerprints**

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Abstract: Molecular markers, protein and DNA fingerprints were used to analyze the genetic variations among three *Rhizobium leguminosarum*. The frequency of genetic variability was detected in three isolates of *R. leguminosarum*. Variation in protein contents (89.25, 92.27 and 90.75 mg/g), protein banding pattern (10, 12 and 11 polypeptide bands) was observed for the three isolates of *R. leguminosarum* M, IS and F, respectively, with 40% polymorphic bands. The variation in peroxidase isozymes was 4, 6 and 5 bands, respectively, with polymorphic bands of 37.5%. Variation in each of protein banding pattern and peroxidase isozymes among the three isolates suggest that DNA fingerprint analysis could be used to show rapid and precise information about genetic variability. Ten arbitrary base primers were successfully used to amplify DNA fragments from three isolates. Three arbitrary 10 base primers (OP07, OP12 and OP18) revealed characteristic fragments where as 20% polymorphic fragments related to total amplified DNA fragments. Monomorphic 48% common amplified fragments as well as unique (genetic markers) 32% for the three *R. leguminosarum* isolates. The frequency of genetic variability (polypeptide, isozymes and DNA) was detected among 3 isolates of *R. leguminosarum* dependent somaclonal variation.

[Mohamed, H.F., Nor El Din, T.A., Abdel-Shakour E.H. and El-Dougdoug K.A. Detection of Genetic Variation Among Three Isolates of *R. leguminosarum* Using Protein, Isozyme and DNA Fingerprints. Journal of American Science 2011;7(8):435-440]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: *R. leguminosarum*; Peroxides isozyme (PRX); SDS-PAGE; RAPD- PCR; Genetic markers Full Text

49 **Geochemical Systematic Exploration of Stream Sediments in Shurchah Area (SE Zahedan)**

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Abstract: Shurchah area is located 60 kilometers to the southeast of Zahedan. Zahedan granitoides and dioritic dykes have intruded in flysches of Eocene age. According to factors such as stratigraphy, lithology, tectonics and topological gravity of drainage patterns, 82 stream sediment samples have been taken from streams. Samples were analyzed using ICP-MS and AAS analytical methods. For eight elements Au, Ag, Cu, Pb, Zn, As, Sb and Hg factors such as error, frequency distribution, amount of sensored, background, threshold, anomaly, mean, mode and standard deviation, calculated individually. Among these elements, Sb with an average value of 10 ppm has been considered as anomaly regarding the spatial situation of the anomaly; it was determined to be in the central part of the study area. In addition, strong positive correlation was observed between gold- arsenic and gold- antimony.

[Mohammad G. Kahrazeh, Hamidoddin Yousefi. **Geochemical Systematic Exploration of Stream Sediments in Shurchah Area (SE Zahedan).** Journal of American Science 2011;7(8):441-449]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: frequency distribution, antimony, anomaly, positive correlation Full Text

50 **The effect of Justice in attraction of customer and for compensate of incomplete services (Case study: Hotels of Shiraz City)**

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Abstract: Successful companies know that right now low price strategy could not provide any guarantee for market leading. Customer satisfaction with regard to high

services and supportive strategies are important tools for pay attention to the requests of customers. It is a need for all companies to focus on customer. Although prevention is so much better than treatment, but receiving no claims is inevitable. Since providing incomplete services is inevitable but pay attention to this matter is so much important for service providers. The present paper intends to consider how much compensate of incomplete services would be effective on customer satisfaction for considering its claim and finally its support of company. Perhaps it is possible to apply it as a marketing tool as well. For this purpose we appointed relevant customers of Shiraz hotels and distributed a questionnaire in order to have the idea of 140 guests who had different claims to the chairman of the hotel. The result was positive effect of justice on prevention from negative consequences of incomplete services.

[Ahmad Askari, Farideh Askari. **The effect of Justice in attraction of customer and for compensate of incomplete services (Case study: Hotels of Shiraz City).** Journal of American Science 2011;7(8):450-458]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: Betterment of Services Process, Procedural justice, Interactional justice, Distributive justice Full Text

51 **Bioaccumulation, Fate and Toxicity of Two Heavy Metals Common in Industrial Wastes in Two Aquatic Molluscs**

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Abstract: Accumulation of chromium (Cr) and cadmium (Cd) by the bivalve *Caelatura (Caelatura) companyoi* and the gastropod snail *Cleopatra bulimoides* was determined. The fate of these metals through soft parts and shells of the molluscs was investigated. Toxicity studies of different concentrations of Cr and Cd on the survival of these organisms were performed. Results showed that both molluscs could accumulate both metals to a large extent and could tolerate their toxicity to high limits, as these metals were accumulated mainly in their soft parts, while small amounts were absorbed by their shells. It could be concluded that the investigated molluscs can be used as bioindicators for pollution of aquatic ecosystems by such heavy metals.

[Moloukhia, H. And Sleem, S., Bioaccumulation, Fate and Toxicity of Two Heavy Metals Common in Industrial Wastes in Two Aquatic Molluscs. Journal of American Science 2011;7(8):459-464].(ISSN: 1545-1003). http://www.americanscience.org.

Key words: Heavy metals, Bioaccumulation, Bivalves, Gastropod snails, Toxicity. Full Text

52 **Comparative Study on the Intestine of *Schilbe Mystus* and *Labeo Niloticus* in Correlation with their Feeding Habits**

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Abstract: The present investigation aims to illustrate the morphological, histological and ultrastructural differences in the intestine of a carnivorous fish, *Schilbe mystus* and a herbivorous fish, *Labeo niloticus*, in correlation with their feeding habits. The histological examination of the mucosa of the intestine of both studied species revealed that it is built up of a simple columnar epithelium that contains goblet cells. Though these, are generally more numerous in the intestine of *Schilbe mystus* than in that of *Labeo niloticus*. The ultrastructural investigation of the mucosa of the duodenum and ileum of *Schilbe mystus* revealed that the enterocytes are tall columnar cells and possess well-developed microvilli, numerous mitochondria, microtubules and numerous lipid droplets. The ultrastructural examination of the mucosa of the ileum of *Labeo niloticus* revealed that the enterocytes are tall columnar and possess well-developed microvilli, numerous mitochondria, microtubules, a few endocytotic channels and aggregated chylomicrons. The enterocytes of the rectum of both studied species are tall columnar and are characterized by the presence of short and well-spaced microvilli, a well-developed vesiculotubular system, numerous microtubules and mitochondria. In addition, the rectal enterocytes of *Schilbe mystus* contain endocytotic vesicles. The intestinal muscularis of both studied species is well-developed and built up of unstriated muscle fibres, which are arranged into two layers: an inner circular layer and an outer longitudinal one. The rectal muscularis of both studied species is thicker than that of the rest of the intestine.

[Fawzy I. Amer; Suzan A. A. Naguib and Fawzia A. Abd El Ghafar Comparative Study on the, Intestine of *Schilbe Mystus* and *Labeo Niloticus* in Correlation with their Feeding Habits. Journal of American Science 2011;7(8):465-484].(ISSN: 1545-1003). http://www.americanscience.org.

Key words: *Schilbe mystus*, *Labeo niloticus,* feeding habits, intestine light and ultrastructure. Full Text

53 **Fuzzy Ideals in CI-algebras**

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Abstract: The fuzzification of ideals in CI-algebras is considered, and several properties are investigated. Characterizations of a fuzzy ideal are provided. **Mathematical Subject Classification:** 06F35, 03G25, 08A30.

[Samy M. Mostafa, Mokhtar A. Abdel Naby and Osama R. Elgendy Fuzzy Ideals in CI-algebras . Journal of American Science 2011;7(8):485-488].(ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Transitive, Self distributive, fuzzy ideal, Upper set. Full Text

54 **Investigating the Role of Total Quality Management Eminence Pattern in Strategic Evolution of Organizations**

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Abstract: This research has been performed in purpose of definition and explanation of Total quality management advanced model and investigating its role in organizations' strategic evolution. This article is in type of developmental researches and its performance method is survey and library studies. Moreover information collecting tool is taking notes. In literature of subject of this article initially the principles of total quality management has been discussed, so that we can obtain better perception in order to applying this system in business and improvement of organizations' performance. In order to apply total quality management in organizations

strategically, we have examined TQM movement in Japan and some cases about using of this method have been mentioned. In fact, in this article the model which has been named Total Quality Management Advanced Model (TQMEX) is based on TQM facts and has been modeled according to instructions of this model. A referendum which has been done in Japan, Hong Kong and South Korea indicates the importance of Japanese system of total quality management advanced model in order to implement total quality management system in organizations. Finally, results and information obtained from this research provide criteria and methods for which are the result of the large organizations' experiences that not only have passed two world's oil crisis and Asian financial crisis successfully, but also have continued their growth while these two crises.

[Lena Yasinian. Investigating the Role of Total Quality Management Eminence Pattern in Strategic Evolution of Organizations. Journal of American Science 2011;7(8):489-495] (ISSN: 1545-1003).http://www.americanscience.org.

Keywords: Total quality management, change management, strategic evolution, financial crisis Full Text

55 **Motivating Lecturers in Iran Teacher Education Centers**

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Abstract: Motivated, healthy, happy, productive, knowledgeable and skillful lecturers increase efficiency in Teacher Education Centers. The main purpose of this research is to survey and represent methods of motivating lecturers. The goal of this study is to examine and to improve method of motivation. Also correlation and comparison are used to indicate rate of links and differences between study variables.The statistical society of this study was all teachers of Teacher Education Centers. Samples were selected non-randomly from each state, which 30% of this sample was examined randomly. Tools for this study were Mazlow's Hierarchy Questionnaires, Maslach Exhaust Questionnaire, and teachers' poll. An ordinal scale was used. The questionnaires were distributed in Teacher Education Centers by the executer et al. Also related documents were used. Descriptive and inferential analysis were used for data analysis. Regarding to five levels of Mazlow's Needs, average of 3.13 was obtained for motivation. This means that lecturers mostly desired "social belong." Average of motivation rate (exhaust rate) was 55.47 (31.69%). This means that lecturers exhaust rate is good with respect to maximum exhaust of 175 (100%). There is no difference for inferential analysis of data between male and female lecturers' view. Motivation level difference between men and women is meaningful, and difference of needs between skillful and unskilled lecturers is meaningful. There are correlations between age and record, age and education, age and exhaust, and record and education. Proposed strategies to improve lecturers' motivation are: 1. increment of salary (15.5%), 2. meritocracy (11%), and 3. appending Teacher Education Center to Ministry of Sciences, Researches, and Technology (10.8%).

[Zohreh Mirsepasi, Saeed Sanei, Mona Mirsepasi. Motivating Lecturers in Iran Teacher Education Centers. Journal of American Science 2011;7(8):496-503]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Motivation; Integral system of Teachers Education; Lecturers Full Text

56 **A review of the validity and reliability of assessment instruments for the final examination for the midwifery students of nursing and midwifery faculty of Mashhad**

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Abstract: According to WHO, the annual mortality rate for mothers is 515000 cases in the world. Most of these deaths can, however, be prevented when skills and knowledge are enhanced in the delivery ward. The poor performance of the delivery supervisors in Iran can be held accountable for most of the mother deaths. The lack of an objective tool reduces the possibility of a systematic and valid evaluation of the clinical examinations particularly at postgraduate programs. Therefore, the present research was conducted to investigate the validity and reliability of assessment instruments for the final comprehensive examination for the midwifery students. In this study, based on the checklist available at the faculty, the students' skills were studied in four test fields (delivery room, clinical gynecologic unit, parental care unit, and mother and children health). Thirty one students (22 from term eight and 9 students from term six) were selected in five consecutive days by two examiners (one of the examiners had no knowledge about the educational level and scientific knowledge of the students). For the face validity and content validity, the experts in the field were consulted. In addition, the criterion validity (the correlation between the score means in the clinical and theoretical courses and the students' total average from the final comprehensive examination) and the construct validity were separately calculated. To calculate the internal consistency, the Chronbach's alpha coefficient and the equivalence reliability (the correlation between the scores was separately reported by the two examiners) were used. The correlation coefficient in the final comprehensive examination of the midwifery program with an average score of 0.22 and 0.52 (p=0.01) for theoretical and clinical courses, respectively proved to be the desirable criterion validity in the assessment forms. Besides, the face validity and the content validity with a high reliability index (0.6-1) were confirmed. The occurrence of high to middle Alpha coefficient reliability (0.51-0.83) in every field of the examination showed an internal consistency in the available checklist. Following the definition of the criteria for the values of the scores, the lowest, and the highest reliabilities were determined to be 0.82 and 73.5%, respectively. This study concluded that the assessment instruments of the final comprehensive examination at the bachelor degree for the midwifery students at the nursing and midwifery faculty in Mashhad, Iran, are recommended as the valid and reliable forms for the assessment of the students at their educational career.

[Nahid Jahani Shoorab , Nahid Golmakani, Seyed Reza Mazluom, Kobra Mirzakhani, Sedighe Azhari, Afsaneh Navaiyan. A review of the validity and reliability of assessment instruments for the final examination for the midwifery students of nursing and midwifery faculty of Mashhad. Journal of American Science 2011;7(8):504-510]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Reliability; Assessment instruments; Clinical skills validity Full Text

57 **Evaluation of Euphorbia Aphylla, Ziziphus Spina-Christi and Enterolobium Contortisiliquum as Molluscicidal Agents**

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Abstract: The present work was carried out to evaluate the molluscicidal activities of ethanoic extract of three medicinal plant species namely *Euphorbia aphylla*, *Ziziphus spina chriti,* and *Enterolobium contortisiliquum* against *Biomophalaria alexandrina* and *Lymnaea cailliaudi* (*nalatensis*) snails the intermediate hosts of schistosomiasis and fascioliasis respectively.the experiments were conducted in accordance with WHO guidelines. Probit analysis was used to determine the $LC_{50}$and LC_{90} after 24 hours exposure. The highest molluscicidal potency was recorded for *E. aphylla*. It exhibited significant molluscicidal activity on both snails' species. The LC_{50} and LC_{90} of this extract against *Lymnaea cailliaudi* were 0.66 and 0.88 ppm respectively and 87.6 and 142.5ppm against *B. alexandrina* followed by *Ziziphus spina- chriti* which showed molluscicidal activity against *L. cailliaudi* with LC_{50} 311ppm and LC_{90} 500 ppm and caused no mortality of *B. alexandrina* up to1000 ppm. The least active was *Enterolobium contortisiliquum* which gave negative results against both snail species up to1000 ppm. Further purification of active compounds present in *Euphorbia aphylla* and *Ziziphus spina- chriti* may eventually be of great value for the control of snails' intermediate hosts of fascioliasis and schistosomiasis.
[Abdalla A. Hassan, Abeer E. Mahmoud, Rasha A. Hassan and Enas A. M. Huseein. **Evaluation of Euphorbia Aphylla, Ziziphus Spina-Christi and Enterolobium Contortisiliquum as Molluscicidal Agents**]. Journal of American Science 2011; 7(8):511-520].(ISSN: 1545-1003). http://www.americanscience.org.

Key words: Plant molluscicides- *Biomphalaria – Lymnaea*- fascioliasis - schistosomiasis -Egypt. Full Text

58 **Perilesional Enhancement Assessment of Hepatic Metastases by Dynamic MRI**

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Abstract: Evaluation of liver metastases is one of the most common indications for magnetic resonance (MR) imaging of the liver. In an era when contrast-enhanced CT and MRI are widely used for the assessment of focal liver lesions, peripheral rim enhancement of lesions on early phase images has been recognized as one of the characteristic findings of metastatic tumors. The purpose of this study is to determine whether perilesional parenchymal enhancement of hepatic metastases was correlated with the degree of tumoral enhancement on arterial phase images or tumor size using dynamic MRI. 67 hepatic metastases lesions in 33 patients were studied for perilesional enhancement and the study findings were high percent of perilesional enhancement at metastases compared to other histologic types of hepatic tumors like hepatocellular carcinoma and cavernous hemangioma. In addition, perilesional enhancement patterns observations showed it was the main component of rim enhancement rather than tumor hypervascular periphery that its size showed inverse correlation with the size of perilesional parenchymal enhancement. Characterizing the metastases from other hepatic tumors as well as different features of perilesional rim enhancement can influence therapeutic planning and an expectation of a better prognosis.
[Yasser Mohamed Fekry. **Perilesional Enhancement Assessment of Hepatic Metastases by Dynamic MRI**. Journal of American Science 2011; 7(8): 521-525].(ISSN: 1545-1003). http://www.americanscience.org.

Key words: perilesional • enhancement • MRI • dynamic • metastases Full Text

59 **The Ethmoidal Ganglion and its Anatomical Relations in Lacertilia (Squamata: Reptilia)**

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Abstract: The ethmoidal ganglion of three lizards belonging to three different families is described. Such ganglion is represented by one part in both *Uromastyx aegypteus* (Family: Agamidae) and *Varanus griseus griseus* (Family: Varanidae) and by two parts in *Sphenops sepsoides* (Family: Lacertidae). In *Uromastyx* and *Varanus* the ganglion lies on the ramus palatinus of the nervus facialis. In *Sphenops*, a small (ventral) part of the ganglion is located on the ramus palatinus while its main (dorsal) part is found on the anastomosing branch between the rami palatinus facialis and nasalis of the nervus trigeminus. In the present study, the ganglion is found at an anastomosing branch between the ramus palatinus of the nervus facialis and the ramus nasalis of the nervus trigeminus in *Sphenops*. In *Varanus* it is found where the ramus palatinus becomes very close to the ramus nasalis, whereas, in *Uromastyx* the ganglion is located posterior to the origin of the anastomosing branch.
[Ahmed Imam Dakrory, and Alaa El-Din Shamakh. **The Ethmoidal Ganglion and its Anatomical Relations in Lacertilia (Squamata: Reptilia).** Journal of American Science 2011; 7(8):526-532].(ISSN: 1545-1003) .http://www.americanscience.org

Keywords: Ethmoidal ganglion – *Uromastyx, Varanus* and *Sphenops*. Full Text

60 **Patient Education: its Effect on Qualiy of Life of Patients with Cancer on Chemotherapy**

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Abstract: Background: Cancer is a devastating disease that can negatively affect a patient's quality of life. Treatment for malignancies in particular, frequently involves complex, intense, and prolonged chemotherapy regimens. Aim of the Work: to assess the effect of educational intervention on quality of life for patients with cancer on chemotherapy. Subjects and Methods: This quasi-experimental study was conducted at the Radiotherapy and Nuclear Medicine Department affiliated to Ain-Shams University Hospitals, on a purposive sample of fifty patients recruited into this study. The inclusion criteria were adult patients, their age ranged between 20-50 years, on chemotherapy; received at least 2-3 sessions of chemotherapeutic agent. The tools included interviewing questionnaire sheet and Quality of Life Index Scale consisting of fo dimensions: Physical, psychological, social and spiritual. The educational intervention was conducted through five sessions each one lasted from 30-45 minutes. Effects of the educational intervention on patient's condition investigated done through comparing the pre, post one month and post six months assessment of the patients regarding the level of knowledge and quality of life. Results: There is highly statistically significant difference in patient's level of knowledge pre, post one month and post six months fro implementation of the educational intervention about cancer regarding to all items. Comparison of patient's quality of life dimensions pre, post one month and post six mont from implementation of the educational intervention revealed there is highly statistically significant differences among them (77.78 ±6.54, 63.54 ±7.85& 50.24±5.47 respectively at $p<0.001$).
Conclusion and Recommendations: Education has highly statistically significant positive effect in increasing the level of patients' knowledge and improving their quality of life. Further investigation could be undertaken to document the cost effectiveness of educational effects in reducing hospital stay, decreasing readmission, improving the

personnel quality of life and minimizing complications of illness and therapies.
[Abeer William Aziz ,Patient Education: its Effect on Qualiy of Life of Patients with Cancer on Chemotherapy]. Journal of American Science 2011;7(8):533-544]. (ISSN: 1545-1003). http://www.americanscience.org.

Key word: Cancer, quality of life, educational intervention Full Text

61 **Characterization of Phospholipase C Productivity by *Pseudomonas aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus* isolates**

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Abstract: Of 205 bacterial isolates, 28 have shown high phospholipase production. Three isolates, identified as *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Staphylococcus aureus*, have shown the highest PLC productivity compared to isolates from the same morphological class and were selected for characterization of enzyme production. PLC production by these isolates took place in phosphate starved media (PS-TMM). For *Pseudomonas aeruginosa* D183, PS- TMM supported maximum PLC production. Replacing glucose with other carbon sources at different concentrations, increasing glucose concentration, adding organic proteins (except for peptone at 0.5% and BSA at 1.5%), or replacing metal salt components of PS-TMM, inhibited PLC production. PS- TMM, to which BSA (1 or 1.5%) or sodium cholate (0.05%) was added, supported maximum PLC production by *Bacillus cereus* D101. PLC production by this isolate was increased with increasing glucose concentration in PS-TMM, while replacing glucose with other carbon sources either decreased or abolished PLC production. Adding BSA, triton X-100, sodium cholate (at 0.05 and 0.1%), tween 20, or tween 80 to PS-TMM or removing $CaCl_2$ from it increased PLC production by this isolate. Production of PLC by *Staphylococcus* isolate in PS-TMM occurred only when glucose concentration was increased to 110 mM, when BSA (0.5, 1 or 1.5%) or sodium cholate (1.25 or 2.5%) was added. PLC production by all three isolates was growth associated. Optimum pH for PLC production by the three isolates was pH 7-8 while optimum temperatures were 37˚C (*Pseudomonas aeruginosa* D183), 30˚C (*Bacillus cereus* D101) and 25-37˚C (*Staphylococcus aureus* D173).
[Nooran Sh. Elleboudy, Mohammad M. Aboulwafa, Nadia A. Hassouna, **Characterization of Phospholipase C Productivity by *Pseudomonas aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus* isolates**. Journal of American Science 2011; 7(8): 545-566]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Phospholipases C, *Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus*, Production, Characterization Full Text

62 **STRUCTURAL AND ULTRASTRUCTURAL ALTERATIONS IN MAMMALIAN ADRENAL CORTEX UNDER INFLUENCE OF STEROIDOGENESIS INHIBITOR DRUG**

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Abstract: The risk of adverse human health effects due to endocrine-disrupting chemicals is of growing concern. In recent years, ketoconazole, an imidazole derivative has been developed and currently used in the medical fields as an anti-fungal and steroidogenesis inhibitor drug. The present study aimed to investigate the influence of ketoconazole in the structural and ultrastructural characteristics of albino rat adrenal cortex. Twenty adult male rats weighing 150-200 g. were divided into two even groups; group I were *i.p.* injected with 10mg/100g.b.wt. of ketoconazole dissolved in *1ml* physiological saline solution in a daily manner at 9am for 15 days. Whereas, group II were injected with *1ml* saline solution in the same manner. *Histologically*, adrenal cortex of treated rat displayed hypertrophy. Glomerulosa, fasciculata and reticularis cells were loaded with lipid droplets of variable sizes, occupying almost the cytoplasm thus displacing the nuclei eccentrically, which showed signs of pyknosis, karyorrhexis and karyolysis. *Ultrastructurally*, the three cortical zones displayed the presence of hypertrophied mitochondria filled with tightly packed tubular cristae, whereas the others having cavitaion results in a complete loss of cristae, and mitochondria are identified by the remainder cristae adjacent to the inner boundaries of the limiting membrane, in addition to extensive accumulation of variable sized lipid droplets and nuclei showing pyknosis and karyolysis. In conclusion, it is noticed that the destructive impacts of ketoconazole on the adrenocortical cells reflected on their functions leading to much deficiency in their performance. So, it should be taken in consideration and great concern that this drug must be utilized under restricted precautions in the medical fields to protect the human health from its hazardous impact.
[Waslat W. Elshennawy and Hanaa R. Aboelwafa. **STRUCTURAL AND ULTRASTRUCTURAL ALTERATIONS IN** MAMMALIAN ADRENAL CORTEX UNDER INFLUENCE OF STEROIDOGENESIS INHIBITOR DRUG**.** Journal of American Science 2011; 7(8):567-276]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: adrenal cortex, histology, imidazole, ketoconazole, rat, steroidogenesis, ultrastructure. Full Text

63 **Inflammatory Allergic Immune Response in Scabies Pyoderma**

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Abstract: Scabies is a highly pruritic disorder induced by an immune allergic response to infestation of the skin by the mite sarcoptes scabiei that burrows in the stratum corneum of the skin. Scabies persists despite the availability of topical insecticides because the disease is transmitted before it is diagnosed and treated and significant resistance to insecticides has developed. The aim of this study was to investigate immunobiochemical response of the host's inflammatory/immune response to scabies during infestation in schoolchildren at rural areas. The number of eosinophil, neutrophil, serum IgE and serum interleukin (IL)-5 were assessed in 21 patients with 20 healthy control subjects. In the present study significantly higher number of eosinophil (11.90 ± 2.0 %vs. 4.12 ± 1.75 %; $p<0.001$) and highly significant elevation in neutrophil (%) in the scabies group compared to the healthy controls (39.9 ± 5.2%vs. 23.5 ± 2.0%, $p<0.001$). There was a highly significant elevation in serum IgE in the scabies group compared to the healthy normal controls (150 ± 58 IU/ml vs. 36.7 ± 18.0 U/ml $p<0.001$). As regards serum IL-5, there was a highly significant elevation in Interleukin-5 in the scabies group compared to the healthy normal controls (44.0 ± 15.3 ug/l vs. 12 ± 3.6 ug/l $p<0.001$).The results of this study suggest that there is a potent inflammatory immune response during the infestation of sarcopties scabiei. In conclusion, scabies is a dangerous disease affecting the immune system and should be treated once discovered to prevent the hazards of being epidemics.
[Mohamed A. Elmaraghy and Abeer M. El Meghawr,y. **Inflammatory Allergic Immune Response in Scabies Pyoderma].** Journal of American Science 2011; 7(8):577-582].(ISSN: 1545-1003). http://www.americanscience.org.

Key Words: Scabies; Eosinophil; IgEl; Interleukin (IL)-5; risk factors of scabies; epidemics. Full Text

64 Oriented Indium Doped Zinc Oxide Thin Films by Spray Pyrolysis Technique

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Abstract: Spray pyrolysis technique has been proved to be an effective tool to produce well oriented undoped and indium doped zinc oxide thin films onto glass substrates. The correlation between the main preparation parameters such as: substrate temperature (673-873 K), spraying time (10-50 min.) and doping concentration (0-3%) and the structural and optical properties of the films, have been discussed. The alignment of the crystals has been investigated by X-ray diffraction, atomic force microscopy and transmission electron microscopy. The prepared ZnO:In films are generally homogeneous, have smooth surfaces and low sheet resistance values R_s in kW range\SONDZEICHEN SYMBOL \f "Symbol" \s 12W. The doping with Indium decreased the sheet resistance of ZnO films by about two orders of magnitude\SONDZEICHEN SYMBOL \f "Symbol" \s 12W.

[M.O. Abou-Helal, **Oriented Indium Doped Zinc Oxide Thin Films by Spray Pyrolysis Technique**]. Journal of American Science 2011; 7(8): 583-587].(ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Zinc oxide, Indium, Spray Pyrolysis, Mono-crystals, Single-crystals. Full Text

65 Histological, Histochemical and Biochemical Studies of the Effect of Chronic Exposure of Noise Stress on the Kidney of Albino Rats

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Abstract: Twenty-five adult male albino rats were utilized to study the effect of noise stress for short and long durations on the kidney structure and functions of albino rats. After one week (short duration) of noise exposure, the biochemical data revealed a significant increase in serum albumin accompanied by a significant increase of body and kidney weights. A significant decrease of urine output was also detected. The renal tufts of the glomeruli were enlarged with increased cellularity filling the Bowman's space. The lining epithelium of the convoluted tubules was slightly swollen. After two weeks of rest, most of the glomeruli and the tubular elements were more or less like those of the control group. After two weeks (long duration) of noise exposure, the biochemical data revealed the same results as those shown in the animals which were exposed to noise for a short duration. However, the body weight of the animals revealed a highly significant increase accompanied by a highly significant decrease in the urine output. The glomerular capillaries of renal corpuscles were swollen, lobulated with increase of the mesengial matrix. The epithelial cells lining renal tubules exhibited cloudy swelling and vacuolar degeneration. After two weeks of rest, the majority of renal glomeruli revealed extensive glomerular retraction and degeneration. The epithelial cells lining the renal tubules showed necrotic changes and their lumina appeared packed with renal casts and cell debris. The thickened basement membranes surrounding both the Bowman's capsules and renal tubules exhibited an intense PAS reaction in rats exposed to long duration of noise stress. After one week of noise exposure, mercury-bromophenol blue staining of the kidney revealed a high protein content in the form of an intense blue colouration of the intact brush borders of the proximal convoluted tubules.

[Mervat S. Zaghloul**. Histological, Histochemical and Biochemical Studies of the Effect of Chronic Exposure of Noise Stress on the Kidney of Albino Rats**]. Journal of American Science 2011; 7(8): 588-598].(ISSN: 1545-1003). http://www.americanscience.org.

Key words: Histological, Histochemical, Biochemical Studies, Noise Stress, Kidney, Albino Rats. Full Text

66 Field Efficiency and its Use for Energy Coefficient Determination

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Abstract: Field efficiency is the most used factor in determining the effective field capacity but yet not known for Iran. This figure for different machines and field conditions are tabulated in ASBAE standards and cited in many publications as a range for different speeds but; they may not hold true for different conditions in different areas. The field efficiency is also needed for converting fuel used in field operation from l/h into l/ha and many more of the kind. Field experiments were run to determine the field efficiency (FE) by measuring wasted time as well as running time for a moldboard plough, chisel packer, tandem disk, mechanical row planter and pneumatic row planter. The energy coefficient for these implements were also obtained and compared. A mean FE of 73.16%, 75.33, 73.5, 68.16, 73.8 and 64.4 and energy coefficient of 26.36, 14.06, 12.19, 6.64, 4.96 and 7.10 l/ha were obtained for moldboard plough, chisel packer, chisel, tandem disk, mechanical planter and pneumatic planter respectively.

[Mansoor Behroozi Lar, Zahra Khodarahm Pour, Mohammad Reza Bamimohammadi. Field efficiency and its use for energy coefficient determination. Journal of American Science 2011;7(8):599-603]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Field efficiency, moldboard plough, chisel packer, tandem disk, energy coefficient Full Text

67 Effect of Forward Speed, Load and Cabin on Tractor Noise and the Health of Drivers

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Abstract: Noise has caused hearing problems to many drivers in the world. It has been investigated that 30 percent of Sweden's farmers suffer from hearing loss. Similar results to those from Sweden were found in a study conducted by University of Iowa in the United States, indicating that American farm workers are faced with the same noise problems in their daily work. A MF399 tractor without cabin and a Valtra 170 with open cabin were tested for noise level at the driver as well as the bystander ear for no load and loaded cases. Comparison were made between noise level in different forward speeds for each tractor with no cabin and open window cabin on driver's ear and bystander for no load and loaded cases. Also Comparison between no cabin and open cabin in each forward speed for no load and loaded tractor at driver's ear as well as at bystander. The noise level at 2.9 km/h for no load open cabin as well as for loaded no cabin and open cabin at driver's ear was significantly greatest among the related speed ranges. It looked like the 2.9 km/h speed with one exception was a threshold after which the SPL (Sound Pressure Level) dropped. For no load no cabin case at the driver ear with a mean value of 87.72 dB(A) no significant difference was observed at 1% level for speeds up to 3.7 km/h; but the SPL for 6.1 km/h was significantly higher. The permissible exposure time was calculated as 4 h/day and 1.9 h/day respectively. For no load open cabin case no significant difference in SPL between SPL at speeds 1.9, 2.2 and 6.1 km/h with a mean value 83.1 dB(A). However significant difference was observed between 2.9 km/h and 3.7 km/h speed. For loaded no cabin, no significant difference was observed between SPL of 1.9, 2.9 and 3.7 km/h with mean value 93.5 dB(A) and 1.1 h/day permissible exposure time. For loaded open cabin. No significant difference between SPL for 3.7 and 6.1 km/h with mean 89.2 dB(A) and permissible exposure time of 5.4 h/day. Also no significant difference between the SPL for 1.9 and 2.2 km/h with mean 83.7 dB(A). Bar graph for all exposure times are shown. The same comparisons were made at bystander's ear and between no cabin and open cabin in each forward speed.
[Mehrzad Payandeh, Mansoor Behroozi Lar, Jahangir Bagheri and Zahra Khodarahm Pour. Effect of Forward Speed, Load and Cabin on Tractor Noise and the Health of Drivers. Journal of American Science 2011;7(8):604-609]. (ISSN: 1545-1003). http://www.americanscience.org.

Keyword: sound level, noise measurements, tractors, cabin, driver's ear Full Text

68 **The influence of stitch length of weft knitted fabrics on the sewability**

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Abstract: The sewing needle penetration force, the feed of the sewing material, sewing needle size are very important parameters during the sewing of knitted fabric. This paper is studying the effect stitch length, yarn count, and needle size on the penetration force which indicates the fabric sewability. Some physical and mechanical properties of these fabrics were also tested. From the results of this study obtained, we can conclude that, the sewability (which is indicated by penetration force) of 100% cotton single jersey fabric is inversely proportional to its stitch length. In the same time the fabric with coarser yarn count gives the higher penetration force. Also we can conclude that, fabrics with coarser yarn counts and shorter stitch length have better functional performance characteristics.
[Z. M. Abdel Megeid, M. Al-bakry, and M. Ezzat, The influence of stitch length of weft knitted fabrics on the sewability. Journal of American Science 2011;7(8):610-617]. (ISSN: 1545-1003). http://www.americanscience.org.

Key Words: stitch length-weft knitted fabric-sewability- abrasion resistance- Air Permeability Full Text

69 **Considering the effect of globalization on ethnic identity of Kurd identity at Sanandaj city in 2010**

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Abstract: This research intends to study any effects of globalization on ethnic identity of Kurd identity at Sanandaj City in 2010. The statistical population of this research is all people more than 15 years old and residing at Sanandaj city (223675) in 2010. We appointed 767 persons as the statistical sample and in accordance with Kukran formula. In theoretical basics of this research we have ethnic identity theory and globalization attitude and any effects of globalization on ethnic identity. The general result is a reduction in any dependency and interests in personal ethnic identity by globalization and obtaining the responses. The result of regression analysis shows that globalization indexes and absorption in world nature (Common royalty, Common interests & Social relations preference) may specify about %.531 of tribal nature variance in this function.
[Samad Abedini. Considering the effect of globalization on ethnic identity of Kurd identity at Sanandaj city in 2010. Journal of American Science 2011;7(8):618-626]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Globalization, Ethnic identity, Common land, Common ceremonies, Common living place, Common language and Common name Full Text

70 **Gastroduodenal Masses: Multidetector Computed Tomography (MDCT) Findings And Differential Diagnosis With Pathologic Correlation**

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Abstract: To study the role of multidetector CT for the evaluation of gastroduodenal masses and differential diagnosis. All patients in the present study (25 patients) were examined with dual slice CT GE using diluted gastrografin oral intake and IV contrast injection of non-ionic contrast agent. The study was included 25 patients suffered from gastroduodenal masses undergoing abdominal US, endoscopic examination, multidetector computed tomography (MDCT) as well as biopsy and histopathological examinations. 20 patients were diagnosed as gastric lesions and 5 patients were diagnosed as duodenal lesions. The patients examined were 17 males and 8 females with the age ranging from 32 to 79 years and the mean age 55 years ± 2SD. Conclusion: Multidetector Computed Tomography (MDCT) has a great value for evaluation and differential diagnosis of gastro duodenal masses with well correlation with histopathological findings.
[Mohammed T. Abdel Hak, Alya Elnaggar and Yasser M. Fekry. Gastroduodenal Masses: Multidetector Computed Tomography (MDCT) Findings and Differential

Diagnosis with Pathologic Correlation. Journal of American Science 2011; 7(8):627-632].(ISSN: 1545-1003). http://www.americanscience.org.

Key words: MDCT • pathology • correlation Full Text

71 **Application of Health Belief Model in Prevention of Breast Cancer among female staffs of Islamic Azad University-Tonekabon branch in Iran**

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Abstract: Background and Objectives: Nowadays, malignancy is considered as one of the most important health burden in the world. In this study, with respect to the importance of training in order to promote preventive behaviors, the application of a training program based on health belief model was surveyed. A semi–experimental study was conducted on 100 female staffs of Islamic Azad university-Tonekabon branch, Mazandaran, Iran. Samples were randomly collected and classified as intervention (case) and control groups by interviewing using a health belief model–based questionnaire. Both groups completed the questionnaire twice within a three months period of time before and after training of intervention group. Data was analyzed by statistical tests and SPSS software (versions 11.5 and16). Based on results obtained by this study, two groups had no significant difference in terms of demographic variables. However, a significant difference was observed among scores of different constructs of this model such as perceived severity, benefits, and barriers before intervention, and scores of knowledge, attitude, perceived benefits and barriers, and act guidance after intervention ($p<0.05$).The findings of this study imply that the present HBM based intervention has had a positive effect on increasing women's knowledge, attitude, and behaviors regarding breast cancer.
[Afsaneh Gity [1], Davood Shojaeizade [2], Abas Rahimi Froshani. **Application of Health Belief Model in Prevention of Breast Cancer among female staffs of Islamic Azad University-Tonekabon branch in Iran.** Journal of American Science 2011;7(8):633-640]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Health Belief Model, Prevention, Breast cancer, Attitude Full Text

72 **MORBIDITY AFTER CHIN GRAFT HARVESTING USING PIEZOSURGERY VERSUS CONVENTIONAL OSTEOTOMY TECHNIQUES**

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ABSTRACT: Augmentation of the resorbed site using chin bone graft is a common method. However, it involves donor site morbidity. Chin graft morbidity involves impaired sensibility in the mandibular anterior teeth, the gingival and chin–lower lip area postoperatively. The aim of this study was to investigate the morbidity at the donor site following harvest of chin bone using piezosurgery versus conventional bone cutting tools. In addition, distances between the bone harvest defect and anatomical structures such as apices of lower anterior ,premolars and mental foramina were analyzed. A group of 20 patients (16 men and 4 women) who had undergone chin bone harvesting for alveolar ridge augmentation or sinus floor elevation procedures were selected. Patients were equally divided into two groups according to the cutting tools used for chin graft harvesting. Group I *(piezosurgery group)*; piezosurgery was used for chin graft harvesting. Group II *(conventional group)*; conventional cutting tools used for chin graft harvesting. The True distances between the donor defect and the apices of the adjacent mandibular teeth were measured on a digital panoramic radiograph. The distance from the lateral margins of the donor defect to the right and left mental foramina (RMF and LMF) was recorded. Sensibility of the lip, teeth, and gingiva was registered one week, one, three, six and 12 months post-operatively. A total of six (30%) patients experienced anaesthesia of the gingivae immediately postoperatively and had full recovery when reviewed at 3 month follow up. Three (15%) of these patients experienced paraesthesia of the chin and lower lip, however full recovery had occurred at the 6- month follow-up. None of the patients reported altered contour or change in profile of the chin area as well as dehiscence of the incision. Three patients experienced pain at the graft site for up to three months postoperatively. Nine patients showed a negative pulp sensitivity reaction in their lower teeth to ethyl chloride at one week postoperatively; this further improved to 5 patients at the 6-month follow-up and to 2 patients at the 12-month follow-up. Parameter comparisons between both piezosurgery and conventional groups showed no statistically significant difference. However, parameters comparisons across each group showed statistically significant difference between different parameters in teeth with and without negative response to thermal pulp testing regarding mean volume of the chin bone defect (P-value < 0.001), mean distance of the defect to mental foramen of both sides (P-value < 0.05). In conclusion, this study indicated that chin graft donor site showed some post-operative morbidity. The most frequent disturbance was impaired teeth sensibility. The lower lip and chin region showed fewer disturbances. However, by correctly placing mucoperiosteal incisions and bone cuts, the risk of sensitivity changes can be minimized. Surgical control for the piezosurgery was easier than conventional methods for mobilizing a chin bone block graft. The force necessary to produce a cut was much less compared to rotational burs. Increased temperature during bone cutting with piezosurgery was avoided which reduces the risk of bone damage as a result of overheating.
[Gamal M. Moutamed. **MORBIDITY AFTER CHIN GRAFT HARVESTING USING PIEZOSURGERY VERSUS CONVENTIONAL OSTEOTOMY TECHNIQUES.** Journal of American Science 2011;7(8):641-652]. (ISSN: 1545-1003). http://www.americanscience.org.

KEY WORDS: autogenous bone, chin bone graft, morbidity, piezosurgery, bone augmentations, sinus floor elevation Full Text

73 **Deformation of Circular Holes at Flanging Process of Thin Sheets**

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Abstract: The theoretical analysis of flanging process of circular holes in thin plates taking into account the interconnected changes of a thickness and deformation hardening is carried out. Possibility of definition of current values of strains in non-stationary processes of forming is shown. In process of flanging the co-ordinate of the material element differentiating area of compression and a stretching in meridinal direction is defined.
Journal of American Science 2011;7(8):653-657]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: Sheet Metal Forming, Thickness Changes, Work Hardening, Flanging Full Text

74 **Toxigenic fungi and aflatoxin associated to nuts in Saudi Arabia**

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Abstract: A survey was carried out in July-Sept., 2009 to obtain data on the occurrence of aflatoxin and the aflatoxins-producing potential of fungi isolated from nuts (almonds, peanuts, hazelnuts, pistachio nuts, Walnut and Cashew) in region of Qassim in Saudi Arabia. The samples were analyzed for aflatoxins by immune affinity column (IAC) clean-up with liquid chromatography and fluorescence detection. Percentages of positive samples with aflatoxins were 80, 80, 60, 40, 40, 20 % for pistachio, peanuts, walnuts, almonds, hazelnuts and cashews. Concentrations of aflatoxin B_1 were ranged between (38- 45, 11- 90, 41 -90, 0.3-3.6, 62-120 and 70-140 " µg/kg") respectively.100% of samples showed variable incidence of fungal contamination. Fungi present in samples were *Penicillium spp, Aspergillus niger*, *A. flavus* and *Rhizopus spp*. Results showed that isolates of *A. flavus* were able to produce aflatoxins B_1, B_2, G_1, and G_2. The purpose of the survey was to determine levels of aflatoxins and to monitor the effectiveness of the controls in place to limit consumer exposure to aflatoxins.
Keywords: exposure to carcinogens, aflatoxins, nuts, HPLC, Fungi, food contaminants**.**
[Mohamed Deabes and Roquia Al- Habib. Toxigenic fungi and aflatoxin associated to nuts in Saudi Arabia. Journal of American Science 2011;7(8):658-665]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Toxigenic; fungi; aflatoxin; nut; Saudi Arabia Full Text

75 **Locating Input/ Output point in Facility Design**

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Abstract: Input and output points location problem is an NP-Hard combinatorial problem with many applications. We consider location of input and output points on perimeter of shortest single loop path. In this paper, a genetic algorithm is developed to solve input and output points location problem. Parameter setting is one of the most important issues of research in Genetic Algorithms (GAs). An efficient experimental design method for parameter optimization in a genetic algorithm was carried out using the Taguchi method. Genetic parameters including the population size, the crossover rate, the mutation rate, gene mutation rate and the stopping condition are considered as design factors. We investigate effect of number of AGV vehicles and their capacity on total time of AGV systems in the uncertain environment. Using simulation based optimization, we determine a robust solution and numerical results show efficiency of our solutions comparing with the result of deterministic approach.
[Hossein Shabazi. Stochastic Location Distribution Problem in A Supply Chain System Locating Input/Output point in Facility Design. Journal of American Science 2011;7(8):666-670]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Input/Output points location; Genetic algorithm; Queuing theory; Simulation optimization; Taguchi method. Full Text

76 **Application of Cost-Benefit Analysis in Assessment of Railroad Projects; a Case Study: Gorgan- Mashhad Railroad (Iran)**

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Abstract: The history of growth and development in developed countries indicate that transportation has an important role in economic growth of these countries, such that most scientists and specialists of economic affairs believe that the transportation industry is the axis of growth and development in Europe and America. In Iran, also, transportation has been introduced as the key to development, and among the different routes of transportation, railroad has an important role in this respect. Considering the large volume of required investment for railroad projects, it is evident that scientific studies are needed to evaluate whether or not these project are economical. In this study, we have used the method of cost-benefit analysis and concluded that establishment of Gorgan-Mashhad railroad is economical.
[Saeed Arabtaheri. **Application of Cost-Benefit Analysis in Assessment of Railroad Projects; a Case Study: Gorgan- Mashhad Railroad (Iran).** Journal of American Science 2011;7(8):671-678]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Cost-Benefit Analysis; transportation; economic; development Full Text

77 **Phonetic Processes in Ruini Dialect**

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Abstract: The article, after introducing the consonants and vowels, discusses the impacts of them on each other as a result of collocation and subsequent changes. The research not only introduces a vanishing dialect, but also it is surely a step forward to protect the cultural heritage of the Persian language. The research method is field-library, in which the researcher has done conversations with different native speakers and residents of Ruyin village. The language corpus consists of native morphemes belonging to the dialect and those items with different pronunciation in contrast to standard Persian.
[Mohammad-Hasan Hatami. **Phonetic Processes in Ruini Dialect.** Journal of American Science 2011;7(8):679-682]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Ruyin; consonant; vowel; phonetic process Full Text

78 **The jurisdiction and discretion challenge of International Court of Justice in Kosovo opinion**

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Abstract: The advisory opinion of the international court of justice (here after ICJ or the court) is a legal constitute which comes from common law system. It is established to aid other bodies of united nation and specialized organizations in their activities according to their statutes. This paper is about to analyses the reasoning of the court on establishment of its jurisdiction and discretion in Kosovo opinion. In this respect, it will consider the question raised by General Assembly or (GA), the articles of charter and statute of the court in this issue, the reasoning of the court and declarations of the judges in favor or against the court reasoning, and the effect of this opinion on development of international law. It seems, this is the first time that the court does not deal with the conduct, rights and obligations of states or international organizations, but rather deals with an action of non- state entities which the legality of their action on issuing the declaration of independence according to international law must be determined.
[Soheyla Koosha. **The jurisdiction and discretion challenge of International Court of Justice in Kosovo opinion.** Journal of American Science 2011;7(8):683-687]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: International Court of Justice; jurisdiction; discretion; Kosovo opinion; advisory opinion Full Text

79 **Change in intramuscular and intermuscular neural adaptation after resistance training in trained college athletes**

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Abstract: The purpose of this study was to investigate the intramuscular and intermuscular neural adaptation after resistance training in trained college athletes. The results indicated that, progressive resistance training significantly (P<0.01) increased MVC and integrated electromyography (IEMG) of MG muscle. There were, however, a significant decrease (P<0.01) in integrated electromyography (IEMG) of TA muscle. It was concluded that, with strength improvement in trained limb agonist muscle activation increased whereas antagonist muscle coactivation decreased. Consequently intermuscular neural adaptation (decrease of muscle coactivation) and intramuscular neural adaptation include increase of motor unit recruitment, firing rate and firing duration, does occur in trained athletes when a new training stimulus is implemented.
[Mansoure Shahraki. **Change in intramuscular and intermuscular neural adaptation after resistance training in trained college athletes.** Journal of American Science 2011;7(8):688-693]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Strength training; electromyography; gastronomies Full Text

80 **The aerial parts of *Stachys schtschegleevii* Sosn. as hydroalcoholic extract has antibacterial Activity on Multi-Drug Resistant Bacterial Isolates in Comparison to Ciprofloxacin**

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Abstract: Several *Stachys* species have been used traditionally for their health benefits in various countries. About 34 species of *Stachys* are found in Iran. This plant possesses various medicinal properties, but there have been performed a few studies on the antibacterial effects of *Stachys schtschegleevii*. The aim of the present study was evaluation of the antibacterial activity of *Stachys schtschegleevii* on 100 Multi Drug Resistant isolates of *Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella spp.* and *Escherichia coli*. *Stachys schtschegleevii* samples were collected from the fields of northwestern Iran. The flowering aerial parts of the plant were cut, and powdered, and then extracted by maceration at room temperature for 72 hours. Bacterial isolates were collected from clinical specimens from several wards of educational hospitals in Urmia, Iran during a 12 months period. The susceptibility of isolates to *Stachys schtschegleevii* extracts was determined using a broth microdilution method. Considering the wide application of ciprofloxacin in treatment of bacterial nosocomial infections, the antibacterial effect of ciprofloxacin on isolates was also determined. All the multi-drug resistant bacterial isolates were sensitive to different concentrations of *Stachys schtschegleevii* hydro-alcoholic extract. The most sensitive bacterial isolates to *Stachys schtschegleevii* extracts were *P.aeruginosa*, however, 69% of isolates were resistant to ciprofloxacin. The results demonstrated that this herbal drug could represent a new source of antimicrobial agents, for the control of hospital acquired infections. However, more adequate studies must be carried out to verify the possibility of using it for fighting these bacteria in human body infections. Additionally, antifungal activity of this plant must be studied along with its antibacterial effect.
[Peyman Mikaili, Nima H. Jazani, Jalal Shayegh, Negar Haghighi, Negar Aghamohammadi, Minoo Zartoshti. The aerial parts of *Stachys schtschegleevii* Sosn. as hydroalcoholic extract has antibacterial Activity on Multi-Drug Resistant Bacterial Isolates in Comparison to Ciprofloxacin. Journal of American Science 2011;7(8):694-699]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: herbal medicine, ciprofloxacin, resistant bacteria, antimicrobials, hospital acquired infections Full Text

81 **Weather types, their frequency and relation with rainfall in west of Iran**

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Abstract: Over time, various places experience different weather; there is even possible and quite tangible some different kinds of weather in a season. In this study, an automated synoptic classification (T S I) with meteorological data of six synoptic stations in the west of Iran has been used, which are exposed to Mediterranean and Sudan systems, in different seasons of the year to categorize each day by its particular category. The used variables include cloudiness (12GMT), maximum and minimum daily temperature, Dew point (12GMT), maximum and minimum daily humidity, dry temperature degree (12GMT), daily rainfall, and u and v components of wind in 1961-2004 periods. After elimination of days lacking data, by creating a P-type matrix, the correlated variables were identified and selected in form of independent components as an objective technique by analyzing principal components, and then a homogeneous collection was extracted from days of record periods in each station under name of

weather types by hierarchical cluster analysis (AHC). Afterwards, through evaluating rainfall amount in every weather type and its related days using cluster analysis (CA), isohyets types were identified in west of Iran. Accordingly, 4 isohyets groups were identified in 26 weather types, including high rainfall, very high rainfall, medium rainfall, and very low rainfall. Occurrence time frequency of weather types in stations of the region was studied in terms of presence number of each weather type throughout the each year and as repeated occurrence in each individual day of statistical year (number of presence of each weather type in 1 Jan of all years then 2 Jan and so to 29 Dec) and subsequently their seasonal activity was determined.
[Ramin Beedle. **Weather types, their frequency and relation with rainfall in west of Iran.** Journal of American Science 2011;7(8):700-707]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Weather type; rainfall; Iran; climate; meteorological Full Text

82 **Considerations over the Biostratigraphy of Jeirud Formation**

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Abstract: Biostratigraphy is the branch of stratigraphy which focuses on correlating and assigning relative ages of rock strata by using the fossil assemblages contained within them. Usually the aim is correlation, demonstrating that a particular horizon in one geological section represents the same period of time as another horizon at some other section. In location of Type section (Jeirud village – north east of Tehran), There are four members (A, B, C and D) and they are with a thickness of 760 meters. Among four members in Jeirud Formation, only member A is pertaining to late Devonian which has been separated, with a clastic unit, with a thickness of 20 meters from other member (B, C and D) in early Carboniferous. Lower boundary of member A is type of parallel unconformity with lilthostraigraphic units (Mila Formation) and is very index but there is no equal opinion about upper boundary. Also, based on advice from national stratigraphy committee in Iran, Jeirud Formation word is only equivalent to member of type section at the present which is representative of upper Devonian rocks in Central and western Alborz B, C, D members in Carboniferous can be compared with Mobarak Formation hence they do not have utility. This article aims to investigate the Considerations over the Biostratigraphy of Jeirud Formation, in addition to the use of basic biostratigraphy in different sediment types.
[Mostafa Yousefirad, Hamideh Noroozpour. Considerations over the Biostratigraphy of Jeirud Formation. Journal of American Science 2011;7(8):708-712] (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Biostratigraphy, Jeirud Formation, Sediment Types, Index Fossils. Full Text

83 **The Study of Some Cognitive and No cognitive Variables as Predictors of School Success and Unsuccessful in the First Year Male High School Students of Ramhormoz**

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Abstract: The purpose of the present study was to investigate intelligence, emotional intelligence, academic self-concept, locus of control and social-economic status as the predictors of academic success and failure of first year high school male students in Ramhormoz. Thus, from all successful students and unsuccessful students 160 persons selected randomly. The materials used in this research are as follows: Petrides & Furnham Emotional Intelligence Inventory, Delavar Academic Self-concept Inventory, Rotter Locus of Control Inventory and a researcher-made Social-Economic Status Inventory. Also Raven's Standard Progressive Matrices score were used from the students personal files. In order to analyze the data both discriminate analysis and descriptive statistics were used. Findings revealed that except component social skills concerned to variable emotional intelligence that was not a predictor of academic success and failure, other variables confirmed the hypotheses of this study and revealed that success or failure male students can be predicted from the variables intelligence, emotional intelligence, academic self-concept, locus of control and social-economic status. The obtained results and the model presented in this study are discussed in detail.
[M. Rashidi, J. Haghighi, M. Shahni Yiylagh, S. S. Hashemi-Sheykhtabatabaei. **The Study of Some Cognitive and No cognitive Variables as Predictors of School Success and Unsuccessful in the First Year Male High School Students of Ramhormoz.** Journal of American Science 2011;7(8):713-723]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Intelligence; emotional intelligence; academic; self-concept; locus of control and social–economic status Full Text

84 **Evaluation of Imprisonment, Good or Bad?**

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Abstract: Nowadays, imprisonment is exerted for the delinquent who commit crimes and do not observe the social, human and moral values throughout the world. It can, without exaggeration, be said that there is no country in the world that has not anticipated the imprisonment in its statutory and does not have prisons and prison wardens because the penal role of the prison is undeniable, and it is impossible for a society to survive and continue without the existence of the prison. Since the late middle ages and renaissance, prison has been used as a punishment. Consequently its usage has reached its peak and has had a very long record in the history. Its history is really deplorable because the prison has been considered as a place so exert a series of punishing measures and/or as a detention house for the accused. But it is different from the organized form of these punishments in the current era because the prisons had not had favorable conditions in the past, and the prisoners were kept in dungeons, castle undergrounds and in the dark and humid places. Thus, many prisoners died of the unfavorable environment of the prisons during their imprisonment.
[Habib Asadi. Evaluation of Imprisonment, Good or Bad? Journal of American Science 2011;7(8):724-737]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Imprisonment, Law, Punishment Full Text

85 **Measuring Islamic-Driven Buyer Behavioral Implications: A Proposed Market-Minded Religiosity Scale**

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Abstract: Islam is one of the major monotheistic religions in the world. Its importance is growing as the number of Muslim adherents is increasing, currently around 1.57 billion worldwide. The wealth of Muslim Arab countries is affecting the global economy as well as the international trade. Since religiosity has a major impact on consumer behavior, it was paramount to have an Islamic Behavioral Religiosity scale, measuring the degree of Muslims' religiosity from the behavioral perspective. The Islamic Behavioral Religiosity Scale (IBRS) could be used by marketers to assess the degree of consumers' religiosity and it could be linked to consumer behavior and work as a predictor to purchase patterns. The scale presented in this paper is a modification to the Islamic religiosity scale presented in 2007 by Chang-Ho C. Ji and Yodi Ibrahim. The scale was modified to include all divisions of Islam; belief, worship, legislation, and morality as well as behavior measurement. The modified scale was evaluated twice. First time was using a sample of 284 Muslim respondents in May and June 2010 and the second time was using 400 Muslim respondents in November and December 2010.
[Mariam Abou-Youssef ; Wael Kortam; Ehab Abou-Aishand Noha El-Bassiouny, Measuring Islamic-Driven Buyer Behavioral Implications: A Proposed Market-Minded Religiosity Scale.Journal of American Science 2011;7(8):728-741].(ISSN: 1545-1003). http://www.americanscience.org.

Key words: Religion,Religiosity, Islamic religiosity scale, Islam Full Text

86 **A study of the effects of an aerobic training program on the social maturity of male non-athlete students: the case of an Iranian experience**

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Abstract: The aim of this study is to evaluate the effect of an aerobic training program on the development of social maturity of non-athlete male students. The research method is semi-experimental. Sixty subjects participated in this study, and were assigned to two groups (test and control). Then by applying a training program (in test group) for a period of 12 weeks, the effect of training program on social maturity was studied. To collect data, a questionnaire which is based on Rao's Social Maturity Scale was distributed before and after the course. The results showed that social maturity rate of the participants was significantly different before and after the training program. Also, it was found that there was a significant difference between control and test groups after training program. A specific sport program can be effective in raising the social maturity level and its triple dimensions.
[Mahdi Majlesi, Gholamreza Shabanibahar, Elahe Azadian.**A study of the effects of an aerobic training program on the social maturity of male non-athlete students: the case of an Iranian experience.** Journal of American Science 2011;7(8):742-747]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Social maturity; Physical activity; Male students; Exercise plan; Aerobics Full Text

87 **Nature and Domain of Islamic Education**

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Abstract: There are three attitudes toward the Islamic education. In the first attitude, the Islamic education is considered as a descriptive system which can be achieved through studying the works of Islamic scholars. In the second attitude, it is an inferential system based on which replies can be provided for all educational problems using the religious texts. In the third attitude, the Islamic education is considered as an inferential-institutional system in which some problems and discussions related to education are extracted from the Islamic texts and some other items are obtained based on empirical methods and religious presuppositions. With a short glance at nature and history of the Islamic education as an academic discipline, this writing tries to survey its research methods and its achievements. (Bagheri, Khosro, 2000)
[Masumeh Alinejad. **Nature and Domain of Islamic Education.** Journal of American Science 2011;7(8):748-752]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Islamic education (existing attitudes in the Islamic education), Islamic education history, research methods in the Islamic education, the relationship of the Islamic education with other educational sciences disciplines Full Text

88 **EAPHRN: Energy-Aware PEGASIS-Based Hierarchal Routing Protocol for Wireless Sensor Networks**

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Abstract. Power management is considered as one of the most critical researching issues in the area of wireless sensor networks (WSN), as it plays the main role in increasing the sensor nodes life time. This paper proposes a new hierarchical routing protocol for stationary wireless sensor networks, called EAPHRN Energy-Aware PEGASIS-Based Hierarchal Routing Protocol for Wireless Sensor Networks (EAPHRN). The proposed scheme attempts to increase both the lifetime and the throughput of the wireless sensor network. The efficiency of proposed protocol is evaluated. The simulation results showed that the EAPHRN protocol can solve the main problems in PEGASIS since it uses a new chain construction algorithm that is completely different that the PEGASIS and is more efficient. It also uses a new chain leader election method that plays a very critical role in the energy saving.
Journal of American Science 2011;7(8):753-758]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Sensor networks, Routing, Lifetime, Energy Full Text

89 **Effect of Forward Speed, Load and Cabin on Tractor Noise and the Health of Drivers**

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Abstract: Noise has caused hearing problems to many drivers in the world. It has been investigated that 30 percent of Sweden's farmers suffer from hearing loss. Similar results to those from Sweden were found in a study conducted by University of Iowa in the United States, indicating that American farm workers are faced with the same noise problems in their daily work. A MF399 tractor without cabin and a Valtra 170 with open cabin were tested for noise level at the driver as well as the bystander ear for no load and loaded cases. Comparison were made between noise level in different forward speeds for each tractor with no cabin and open window cabin on driver's ear and bystander for no load and loaded cases. Also Comparison between no cabin and open cabin in each forward speed for no load and loaded tractor at driver's ear as well as at bystander. The noise level at 2.9 km/h for no load open cabin as well as for loaded no cabin and open cabin at driver's ear was significantly greatest among the related speed ranges. It looked like the 2.9 km/h speed with one exception was a threshold after which the SPL (Sound Pressure Level) dropped. For no load no cabin case at the driver ear with a mean value of 87.72 dB(A) no significant difference was observed at 1% level for speeds up to 3.7 km/h; but the SPL for 6.1 km/h was significantly higher. The permissible exposure time was calculated as 4 h/day and 1.9 h/day respectively. For no load open cabin case no significant difference in SPL between SPL at speeds 1.9, 2.2 and 6.1 km/h with a mean value 83.1 dB(A). However significant difference was observed between 2.9 km/h and 3.7 km/h speed. For loaded no cabin, no significant difference was observed between SPL of 1.9, 2.9 and 3.7 km/h with mean value 93.5 dB(A) and 1.1 h/day permissible exposure time. For loaded open cabin. No significant difference between SPL for 3.7 and 6.1 km/h with mean 89.2 dB(A) and permissible exposure time of 5.4 h/day. Also no significant difference between the SPL for 1.9 and 2.2 km/h with mean 83.7 dB(A). Bar graph for all exposure times are shown. The same comparisons were made at bystander's ear and between no cabin and open cabin in each forward speed.
[Mehrzad Payandeh, Mansoor Behroozi Lar, Jahangir Bagheri and Zahra Khodarahm Pour. Effect of Forward Speed, Load and Cabin on Tractor Noise and the Health of Drivers. Journal of American Science 2011;7(8):759-765]. (ISSN: 1545-1003). http://www.americanscience.org.

Keyword: sound level, noise measurements, tractors, cabin, driver's ear Full Text

90 **Investigation of Micro Facies and Renovation of Old Formation Environment in Central Iran**

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Abstract: The formation of Central Iran includes sequences of Dolomite, Dolomite ironstone and ironstone to upper Sanpaleosen-Ethosen in Zagros Mountains. For identification of micro facies of this formation from 216 m of its thickness in Dodaj Cutting (West of Shiraz) 120 thin sections was investigated. Performed desert and laboratory studies resulted to recognition of 7 micro facies and since this deposition is formed of benthic foraminifers, therefore by using of these suitable indices as a valuable tool, we can determine old environments of these deposits. Lack of turbidity, damming and reify and other studies show that Jahrom is formed in an ideal carbonate ramp. Because of high variety and plentifulness of benthic foraminifers, also this carbonate ramp is known as "Carbonate ramp system with plentifulness of benthic foraminifers".
[Mostafa Yousefirad, Hamideh Noroozpour. Investigation of Micro Facies and Renovation of Old Formation Environment in Central Iran. Journal of American Science 2011;7(8):766-768] (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Micro Facies, Central Iran, Old Formation Environment, Renovation. Full Text

91 **Investigation of Effective Factors on Audit Committee Effectiveness from Perspective of Independent Auditors and Board Members**

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Abstract: The mere presence of the audit committee does not necessarily translate into an effective monitoring body. As a result, the search for mechanisms to enhance corporate governance and increase the quality of financial reports has mostly focused on the structure of audit committees. The purpose of this research is an investigation of effective factors on audit committee effectiveness from perspective of independent auditors and board members. The statistical survey of two groups of independent auditors and board members of companies listed on the stock exchange. Data was collected using a questionnaire. 269 questionnaires from the independent auditors and board members of 347 questionnaires were analyzed. Results of this study showed that independence, activity, financial expertise, size, chart, support of board of director and experience caused the audit committee effectiveness.
[Mehdi BaharMoghaddam, Tabandeh Salehi. Investigation of Effective Factors on Audit Committee Effectiveness from Perspective of Independent Auditors and Board Members. Journal of American Science 2011;7(8):769-775] (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Audit committee, Effectiveness, Independence auditor, Board of director Full Text

92 **SOCIAL CAPITAL DIMENSIONS AND FOOD SECURITY AMONG FARMING HOUSEHOLDS IN OGUN STATE, NIGERIA**

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ABSTRACT: Despite an annual 2.5% growth in food production in Nigeria, food insecurity at the national and household level is dismal and on the increase from 18% in 1986 to 40% in 2005. With several studies on social capital and welfare in Nigeria, there is a dearth of information on the role that social capital plays in mitigating food insecurity. This study examined effects of social capital on food security among farming households in Odeda LGA of Ogun state, Nigeria. A multistage sampling technique was used to select 116 households in the study area in 2010. Data analysis was done using descriptive statistics and Probit regression. The food security line was ₦2,155.74 per month per adult equivalent. Based on this, 45% of the total sampled households were food secure while 55% were food insecure. Food secure households exceeded the calorie requirements by 19% while food insecure households fell short of calorie requirements by 28%. A unit increase in social capital ($p<0.01$) increases the probability of household to be food secure by 0.0991 while a unit increase in household size ($p<0.01$) and level of education of household head ($p<0.01$) decreases the probability of household to be food secure by 0.3482 and 0.1367 respectively. Disaggregation of social capital into its dimensions shows that cash contribution positively and significantly affects food security of farming households. Consistent with our prior expectation, households with higher levels of social capital are less likely to experience hunger.
[ONI, Omobowale Ayo. **SOCIAL CAPITAL DIMENSIONS AND FOOD SECURITY AMONG FARMING HOUSEHOLDS IN OGUN STATE, NIGERIA.** Journal of American Science 2011;7(8):776-783] (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Social Capital, Food Security, Farming Households, Ogun State Full Text

93 **Explaining the Implications of Climate Change and the Associated Farmers' Risk Preferences in Nigerian Agricultural Economy**

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Abstract: Climate change is a change in the statistical distribution of weather over periods of time that range from decades to millions of years. Agriculture however, is extremely vulnerable to climate change. Higher temperatures eventually reduce yields of desirable crops while encouraging weed and pest proliferation. Managing climate risk is especially important in agriculture not only for the direct impact that climate has on production, but also because most farmers tend to be risk averse. The fore knowledge that farmers' are mostly risk averse therefore calls for re-opening of an enabling policy environment that effectively considers the farmers perception of risk. This therefore provides a basis for making policy option towards adaptation to climate change given their characteristic socio-economic environment.
[Salimonu, K.K. **Explaining the Implications of Climate Change and the Associated Farmers' Risk Preferences in Nigerian Agricultural Economy.** Journal of American Science 2011;7(8):784-787] (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Implication; Climate; Farmer; Nigerian; Agricultural Economy Full Text

94 **Frequency of Distribution of Interleukin 6 Gene 174G/C Polymorphism in obese Egyptian Cohort**

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Abstract: Elevated IL6 level is documented in obesity. 174G/C polymorphism in the promoter area of IL6 gene may alter its expression or its effect. Aim: Finding the frequency of IL-6 174G/C polymorphism, its sex distribution and its association with obesity and dyslipidemia. Subjects and methods: This study included 74 apparently healthy subjects (45 men and 29 women). BMI was calculated. Lipid profile was assessed by specific colorimetric assays. Serum IL6 level was measured by ELISA. IL6 genotyping was done by PCR-restriction fragment length polymorphism. Results: According to IL6 polymorphism, subjects were classified into 3 groups; CC, GC and GG. 71.42% of the GG group and 68.97% of the GC group were male. Similarly, 78.57% of the GG group and 72.41% of the GC group were obese. No significant difference as regarding sex or BMI was found in the CC group. No significant difference was found among the 3 groups as regarding IL6 level. According to BMI subjects were further classified into Obese (more than 25Kg/m^2) and Non obese (less than 25Kg/m^2) .IL6 and triglyceride levels were higher in the obese group while HDL cholesterol was higher in the non-obese group. Both GG and GC genotypes showed significant positive correlation of IL6 with BMI (P=0.024 & P=0.012 respectively). Moreover, the GC group showed a significant positive correlation between IL6 and LDL cholesterol (p=0.022). CC genotypes didn't show any correlation of IL6 with either BMI or any measure of the lipid profile. Conclusion: The G containing alleles, GG & GC carriers are prevalent in male Egyptians which make them more vulnerable to obesity and its deleterious outcome than CC carriers.
[Riham G.Mahfouz , Azza M. Abdu Allah, Seham A. Khodeer, Waleed F. Abd Elazeem, Mostafa Al Nagar and Walid A. Shehab-Eldin, Frequency of Distribution of Interleukin 6 Gene 174G/C Polymorphism in obese Egyptian Cohort. Journal of American Science 2011;7(8):788-794] (ISSN: 1545-1003).
http://www.americanscience.org.

Keywords: Interleukin 6, Polymorphism, Obesity, Dyslipidemia. Full Text

95 **CODE NO. 6772am_20110820**
BACTERIOLOGICAL QUALITY ASSESSMENT OF WATER HAWKED IN SOME PARTS OF MUBI METROPOLIS, ADAMAWA STATE, NIGERIA

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Abstract: Investigation of bacteriological quality of drinking water hawked around five different areas in some parts of Mubi town in Adamawa state, Nigeria was conducted, using most probable number (MPN) technique and standard plate count methods. Two samples were collected from each site (one at the source and the other from hawkers at the point of delivery to house holds) which was repeated weekly for a period of one month. Mesophilic counts for the samples at the point of collection had values> 10^3 cfu/ml. whereas only two samples (Shuware and Lokuwa at the point of delivery) showed values = 10^3 cfu/ml. However, neither at the source nor at the point of delivery showed MPN values within acceptable range of <1O/IOOml. Therefore, the findings recommend improvement in sanitary quality at the source and enlighten the hawkers on measures that reduce microbial contamination during handling.
[Bashir sajo mienda. Bacteriological Quality Assessment of Water Hawked in some parts of Mubi Metropolis. Journal of American Science 2011;7(8):795-797]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: Bacteriology; water; quality; hawkers; Mubi Full Text

96 **Designing Reinforced Concrete Frames with Earthquake Damage Control**

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Abstract: Controlling the amount of structural damage is one of the most important issues in new design methods such as performance-based design. The purpose of present research is to present a new design method with damage control for reinforced concrete bending frames. For this purpose, at first a static damage standard is developed and then suggested method is applied to design a 7 store frame. Then in order to assess suggested method accuracy, non-elastic damage analysis is performed on mentioned frame. The results show that suggested method is effective on designing reinforced concrete bending frames, with damage control.
[Mehrdad Dorvaj. Designing Reinforced Concrete Frames with Earthquake Damage Control. Journal of American Science 2011;7(8):798-803] (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Damage, performance-based design, Bending frame, Reinforced concrete, non-elastic analysis Full Text

97 **Mechanical Properties of Acrylonitrile butadiene/ Ethylene Propylene Diene Monomer Blends: Effects of Blend Ratio and Filler Addition**

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Abstract: Blends based on acrtlonitrile butadiene rubber (NBR) and ethylene propylene diene monomer (EPDM) rubber was prepared with different fast extrusion furnace black (FEF) concentrations. The effect of blend ratio and FEF contents on the mechanical properties, such as stress-strain behavior, tensile strength and elongation at break have been investigated. Tensile and elongation at break showed synergism for the blend containing zero NBR/100EPDM and 80 phr FEF-content. The experimental data have been compared with the relevant theoretical models.
[S.A. AL-Gahtani, Mechanical Properties of Acrylonitrile butadiene/ Ethylene Propylene Diene Monomer Blends: Effects of Blend Ratio and Filler Addition. Journal of American Science 2011;7(8):804-809] (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: NBR; EPDM; Rubber blend; mechanical properties Full Text

98 **Reducing the Amount of Mineral Nitrogen Fertilizers for Red Globe Grapevines by Using Different Sources of Organic Fertilizers**

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Abstract: This investigation was conducted for three successive seasons (2009, 2010 and 2011) in a private vineyard located at El-Khatatba, Menoufiya governorate; on mature Red Globe grapevines to study the possibility of reducing the amount of mineral nitrogen fertilizers for Red Globe grapevines by using different sources of organic fertilizers. The chosen vines were five years old, grown in a sandy loam soil, spaced at 2 X 3 meters apart, irrigated by the drip system, and cane-pruned and trellised by the Spanish Parron system. Ammonium sulphate 20.5% was added as a source of mineral fertilization. Organic fertilizers (compost and chicken manure) were applied either in the form of individual or mixed at 1:1 ratio on the basis of organic manure content of nitrogen. Thirteen treatments were applied as follows: 100%mineral nitrogen (control), 75%mineral nitrogen+ 25%compost, 75%mineral nitrogen + 25%chicken manure, 75% mineral nitrogen + 25%mixed manure, 50%mineral nitrogen + 50%compost, 50% mineral nitrogen + 50%chicken manure, 50% mineral nitrogen + 50%mixed manure, 25% mineral nitrogen + 75%compost, 25% mineral nitrogen + 75%chicken manure, 25%mineral nitrogen + 75%mixed manure, 0%mineral nitrogen + 100%compost, 0% mineral nitrogen + 100%chicken manure and 0% mineral nitrogen + 100%mixed manure. The results revealed the possibility of using organic nitrogen fertilizers as a partial substitute of mineral nitrogen fertilizers. This study indicated that application of 50%mixed manure included compost and chicken manure combined with application of 50%mineral nitrogen fertilizer was the best management system for achieving the best yield with its components as well as the best physical properties of bunches, improving the physical and chemical characteristics of berries, reducing nitrate and nitrite content of berries and ensuring the best vegetative growth parameters, leaf chlorophyll and leaf mineral content of Red Globe grapevines.
[Abd El-Wahab, M.A. **Reducing the Amount of Mineral Nitrogen Fertilizers for Red Globe Grapevines by Using Different Sources of Organic Fertilizers.** Journal of American Science 2011;7(8):810-819] (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Grape, Red Globe, Mineral, Nitrogen, Fertilizer, Compost, and Chicken manure Full Text

99 Power, Cooperation, Trust and Commitment in Supplier-Buyer Relationships

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Abstract: Facing rapid changes in technology and globalization of markets, firms become very difficult to live alone. The cooperation between buyer-supplier helps to improve the efficiency of the supply chain as a whole for the mutual benefits of both parties involved. Supplier's exercising power is a critical factor that influences cooperation. This study aims to investigate how supplier's use of power affects cooperation climate between a buyer and a supplier, buyer's trust in the supplier, and buyer's commitment from buyer's perspective. Their relationships are hypothesized and investigated based on the empirical data collected from companies in the Iran Cosmetics industry. The results demonstrate that use of non-coercive power improve cooperation and trust between the buyer and supplier. The buyer is inclined to make commitment to the supplier when cooperation and trust exist. Practical insights are provided accordingly in this paper to achieve closer buyer-supplier relationships in nowadays highly competitive environments.
[M. Abolhasanpour, M.M. Seyed Esfahani, M.A. Kimiagari, A. Abolhasanpoor, M. Tajalli. Power, Cooperation, Trust and Commitment in Supplier-Buyer Relationships. Journal of American Science 2011;7(8):820-826] (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: exercised power, cooperation, trust, commitment Full Text

100 **Multiagent Architecture for Management of Milk Tankers in Dairy Industry**

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Abstract: In the business sector there has been and always will be a demand for a single master mind or an expert which can look after the crucial business activities round the clock throughout a year. This is not possible for any single human being as the business activities are either controlled by more than one human in the form of a team or by a single human in a discontinuous manner. This introduces an inconsistency in the decision making process. There has always been search for artificial methods to perform the same task in a consistent manner. This research paper has explored the way how intelligent multiagent system can be used to look after such activities in real time. In this paper the architecture of MAS for the management of milk tankers for a milk processing company is discussed. It involves a combination of knowledge base deduction methods with multi agent techniques, working together to accomplish this task. The financial aspects of the project are not included in the scope of this research.
[Aslam Muhammad, Ahmad Zargham, Martinez-Enriquez A. M., Multiagent Architecture for Management of Milk Tankers in Dairy Industry. Journal of American Science 2011;7(8):827-832] (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Artificial Intelligence; Multiagent system Full Text

101 Intelligent Assistant to Help Blind people for Selecting Wearable Items

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Abstract: We introduce intelligent assistant for the blind people that support them in selecting wearable items. Many intelligent systems have been developed for the people with impaired vision. These systems help them to deal with daily life problems like avoiding obstacles, face recognition, reading, and writing. However, blind persons still have to face a lot of problems in choosing their daily commodities .Therefore a system is needed for such people that can assist them in selecting their accessories. Our proposed system that is using text to speech technology for direct communication is intelligent enough to facilitate blind people in choosing their wearable items like dresses, shoes, hair styles, jewelry, and glasses according to the causal or formal events. The main purpose of this assistant is to make blind persons more independent and more confident so that they can enjoy life like healthy persons. The system's objectives are achieved with the unification of artificial intelligence particularly knowledge based system and image processing. We validate our system on various blind persons and get satisfactory results. [**Farzana Jabeen, Aslam Muhammad, Maliha Saleem Bakhshi, Martinez-Enriquez A. M**. Intelligent Assistant to Help Blind people for Selecting Wearable Items. Journal of American Science 2011;7(8):833-840] (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: vision impaired, text to speech technology, Blind assistance Full Text

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Automatic Generation of Extended ER Diagram Using Natural Language Processing

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Abstract: Extended Entity Relationship Diagrams are an important step in information system design and software engineering. In the early seventies Peter Chen developed an efficient database management system, the ERD. Later on, ERD was enhanced to Extended ERD by adding new concepts like generalization and specialization. The inspiration of EERD emerged from the common need to many organizations to have a unified methodology for file structure and database design. To meet the demands of users, to interpret problem statements in English, applying all the rules and generating an EERD. The structural approach is used to parse the sentences and tag them into different parts of the speech. This is because a belief has been developed that semantics can be completely represented in structures. The structural approach is used to map the tagged words into entities, attributes and relationships. [Dr. Muhammad Shahbaz, Dr. Syed Ahsan, Muhammad Shaheen, Rao Muhammad Adeel Nawab, Syed Athar Masood. Automatic Generation of Extended ER Diagram Using Natural Language Processing. Journal of American Science 2011;7(8):1-10]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Geographic Information System (GIS), Usability, Interactivity, Human-GIS Interaction, Positional Accuracy, Hydrocarbon Exploration, Backpropogation Neural Network

1. Introduction

Databases today have become indispensable to almost any business carried out by an organization. So why not let Artificial Intelligence use its expert systems to handle the entire progression of construction - starting from a simple textual user input to the generation to EERDs (Extended Entity Relationship Diagram)? Application of structural analysis for the generation of EERD is something unprecedented in the history of Artificial Intelligence and Database Designing. Research along similar lines has been done previously but never ever has such a project been implemented.

During the stream of this project we have taken up the task of applying Structural Analysis to create the EERDs that could be further used to generate the tables in accordance with the normalization rules and keeping the functional dependencies intact. This would involve categorizing the parsed input as nouns, verbs and adjectives - a form that could be transformed and identified specifically as entities, relationships and attributes for the EERD. After the analysis and documentation phase we plan to implement the project along the following modules.

Module 1: Reading and parsing natural language input text given by the user.

Module 2: Heuristically classifying the text, that would serve as input to our next module.

Module 3: Generation of ERD and the final output in the form of a graphical diagram.

The third module is however mostly concerned with the generation of a text file that contains all the information needed to generate the ER diagram. This file would then be converted into a format that can be imported to an external tool. In our case the external tool is DeZign. In short the problem statement is very simple. Input in English language and the output is the desired ERD.

What is Conceptual Modeling

Conceptual modeling is a very important and powerful step in relational database design. It overcomes several restrictions of the relational model. The orientation of current relational technology has led to several problems of database modeling and design. For instance, the following restrictions and problems can be solved if conceptual modeling approaches are used:

Normalization is mainly an optimization of structures. Given a set of integrity constraints, the enforcement or maintenance of these constraints has to be programmed. For instance, functional dependencies cannot be represented by constraints defined in relational DBMS. Such constraints are also the reason for anomalous behavior of update functions. Normalization aims now in restructuring database relations through decomposition in such a way that the only constraints, which have to be added to the structure, are those which are based on the DBMS. However, normalization does not take into account the behavior of the database itself. For instance, if operations, which are used often in the application, require the consideration of several

relations and for this reason the performance of the DBMS for such operations is too low, then the solution is to compose those relations again into one relation. This process is called 'denormalization'.

'Denormalization' can also be required after restructuring or extending the database. Since the normalization process is an optimization process independent of the DBMS and since the process is supported by algorithms, the normalization of conceptual models ofthe reality can be incorporated into the translation process. From the other side, normalization can be performed already on the conceptual level. Therefore, structural optimization and behavioral optimization can be treated consistently during conceptual modeling if a powerful proof method is used during optimization. Conceptual modeling has been understood for a long period as modeling of structures and static integrity constraints. Because some powerful structural constructs have been developed and used in practice, a belief has been developed in the community that semantics can be completely represented by structures. Based on this belief it has been assumed that application programming can do the rest and that triggers can be used without problems. Later, it has been discovered that triggering is only safe under certain hierarchy conditions. Therefore, the current thinking is that conceptual modeling should integrate modeling of structures and behavior at the same time.

The same application can be modeled by different models. These models can be equivalent. Since the ER model has a powerful theory behind it, we can consider different models at the same time for different user groups and map these models to each other. The other model can be considered to be a view of the first. The same observation can be made for multidimensional databases and OLAP applications. The star and the snowflake schemata (used in data warehousing and workflow applications) are views on the conceptual schema. Whether views are materialized as it is the case in multidimensional databases depends on the application and on the complexity of the view generation. Refer to [7].

Relationship b/w Natural Language & Conceptual Modeling

It is now understood that that conceptual models have their root in the phrasal form of natural languages. The observation has been made for the sentence construction of the English language as well as for the more complex constructions used in other languages such as German It has been shown that the basic primitives in the sentence construction and the grammar of the English language are very similar to the primitives in ER diagram technique. Because of this similarity, it is conjectured that conceptual modeling could be as powerful as natural languages as a tool for modeling the reality. Current research shows that approaches such as ellipses, ambiguity, changes in semantic meaning can be expressed through constructs developed for conceptual modeling. As a result of this type of research activities, conceptual models now can describe the reality more formally and with well-defined semantics specifications made on the basis of natural languages. Linguists treat semiotics as consisting of three parts: syntax, semantics and pragmatics. Syntax defines the rules for forming sentences. Semantics is concerned with the meaning of words and sentences. Pragmatics deals with practical results, reasons and values. Computer scientists are often mainly concerned with syntax, only partially concerned with semantics and very seldom concerned with pragmatics. Conceptual modeling is based on a certain syntax, which has to have a well-specified meaning. It also has to deal with pragmatics. For this reason, a well-founded theory of conceptual modeling has been extended by methodologies for modeling [7].

[12] develops a dialogue tool with in a big project i.e. RADD (Rapid Application Development). The dialogue tool takes the input from the user in natural language, sample data is used to find out the semantic constraints on the database to be built. This work focuses on implementing the semantic constraints as it is the prerequisite for the normalization and denormalization or any other restructuring approach. Semantic constraints are important because they are necessary for the efficient and effective working of the database.

Some new heuristics were proposed by [13]that assist the semi-automated generation of Entity-Relationship (ER) diagrams for database modeling from a natural language description. The work done by [13] revises the correspondences between the English structure and extended entity relationship diagram. Some new features have also been added as the [8] woek only discusses the basic ER diagram constructs.

Differences between Data Modeling and Database Design

It is worth while to distinguish between Data Modeling and Database Design before discussing the various tools that are available in the market - on the internet - for the former. The differences between the two activities are highlighted as

follows:
For data modeling, the question being asked is:
"What does the world being modeled look like?"
In particular, one is looking for similarities between things. Then one identifies a 'supertype' of some thing which may have sub-types. For example:
• Customers ('super-type') will have Corporate Customers and Personal Customers ('sub-types').
• If supplier contacts are conceptually different things from customer contacts, then the answer is that they should be modeled separately. On the other hand, if they are merely 'sub-sets' of the same thing, then model them together.
On the other hand, for database design, a different question is being answered, altogether:
"How can one efficiently design a database that will support the functions of proposed application, Web Site etc.?"
The key task here is to identify similarities between entities so that one can integrate them into the same table, usually with a 'Type' indicator.
For example:
• A Customer table, which combines all attributes of both Corporate and Personal Customers.
As a result, it is possible to spend a great deal of time, breaking things out when creating a Data Model, and then collapsing them back together when designing the corresponding database.
Some of the most common Data Modeling techniques used today in the fields of Object Oriented Design (OOD) and Software Engineering (SE) are the System Sequence
Diagrams (SSDs), Data Flow Diagrams (DFDs), Use Case Diagrams (UCDs) and UML Diagrams, to name a few.
Entity Relationship Diagram (ERD) is just another representation of Data Modeling employed for designing databases. Various tools and software are available that assist in the drawing and diagrammatic illustration of ERDs. These tools are not responsible for automated generation of ERDs, but rather provide a platform for the user to graphically represent the information using various symbols. Some of these tools go as far ahead as to correct the mistakes that the user may have made in the course of drawing. Moreover, it is also possible to import the ERDs drawn further, to soft-wares that design the database from the respective ERDs. It should be noted here that there can be many representations of ERDs of the same problem statement. Similarly the database generated or designed can also vary from software to software for the same specific scenario. Hence one problem statement may lead to several different databases (i.e. different structures of databases) depending on the number of choices that one has in the intermediary steps.

Entities
The basic object that the ER model represents is an entity, which is a thing in the real world with an independent existence. An entity may be an object with a physical existence - a particular person, car house pr employee - or it may be an object with a conceptual existence - a company, a job or a university course. Each entity has attributes - the particular properties that describe it. For example an employee entity may be described by the employee's name, age, address, salary and job. A particular entity will have a value for each of its attributes. The attributes values that describe each entity become a major part of the data stored in the database. Several types of attributes occur in the ER model: simple versus composite (composite attributes can form a hierarchy), single-valued versus multi-valued; and stored versus derived (some attributes values can be derived from related entities). There is also a null value of an attribute. An entity type defines a collection (or set) of entities that have the same attributes. Each entity type in the database is described by its name and attributes. An entity type usually has an attribute whose values are distinct for each individual entity in the collection. Such an attribute is called a key attribute. Some entities have more than one key attribute [1, pp-41-111].

Relationships
Whenever an attribute of one entity type refers to another entity type, some relationship exists. A relationship type R among n entity types defines a set of associations - or a relationship set - among entities of these types. As for entity types and entity sets, a relationship type and its corresponding relationship set are customarily referred to by the same name R. In formally, each relationship instance is an association of entities, where the association includes exactly one entity from each participating entity type. Each such relationship instance represents the fact that's the participating entities are related in someway in the corresponding mini-world situation. In ER diagrams, relationship types are displayed as diamond shaped boxes, which are connected by straight lines to the

rectangular boxes representing the participating entity types. The relationship name is displayed in the diamond-shaped box [1, pp-41-111].

Cardinalities

The cardinality ratio for a binary relationship specifies the number of relationship instances that an entity can participate in. the possible cardinality ratios for binary relationship types are 1: 1, 1: N, N: 1 and M: N. Cardinality ratios are displayed on ER diagrams by displaying 1, M, and N on the diamonds that represent the relationship type[1, pp-41-111].

Analysis and Design

The analysis and design of our project is the very next step that is followed after a thorough understanding of the nature of the problem is achieved. This is the process of establishing the services the system provides and the constraints under which it must operate to the optimal level of performance. Firstly, it is the identification and description of the use cases. The interaction of these use cases is depicted in the use case diagram. Then for each use case, a system sequence diagram is drawn. This shows the user - system interaction. The responses by the system and the user to each other's events are illustrated here. System sequence diagram contracts are written to further elaborate the complex functions operating in the system sequence diagram. Similarly, collaboration diagrams are drawn for each system sequence diagram demonstrating the interaction between the classes through appropriate functions. Next all the concepts are identified followed by their attributes. This leads to the conceptual model. Secondly the functions within each concept are recognized which leads to the design class diagram.

Secondary Goal

The primary objective of this system is primarily defined in the overview statement i.e. the automated generation of an Extended Entity Relationship Diagram (EERD) through Structural Analysis. However secondary goals that could be achieved from this tool are as follows:

- The ERD generated would be exported to an external tool for further modification and correction.
- The backend tagging of the parsed words to different parts of speech could be used for similar purposes that require classification of words.

Implementation Details

In many information systems projects, requirements are primarily documented in English, and then database designers convert these English descriptions into database schemas in terms of ERDs. During the course of this project we have proposed a number of rules to generate an ERD diagram from English sentence structure. The basics constructs of English such as noun, verb, adjective, and adverb are found to have counterparts in the ERD. Finally and example is used to demonstrate the applicability of these rules in database design.

User Interface -Input Problem Statement

The program begins with the user interface as shown in Figure 2. User enters information and information is processed to generate an ERD using structural analysis approach. The front end input screen consists of six buttons each of which has a specific task which has been explained below.

Clear Text

When the user clicks on this button any previously written problem statement in the text field is erased. This is done so that the user can write a new problem statement.

Format Input

The user types the text in the input field shown as the white text field. The text entered by the user in the text field is formatted so that it can be send to the backend to be processed and finally generate an ERD. A space is put between full-stops and commas. Each sentence ends with a full stop.

Import:

The user has two options, he can either input problem statement directly into the text field by clicking on the button 'enter text', or he can import a previously stored problem statement from any directory in the computer.

When the user clicks on import the text field appears on the screen, this is where the imported file is displayed. To import a file it is essential for the user to give the destination of the file. One constraint to this is that the file has to be in either .txt or .rtf format to be imported.

Submit

Each sentence ends with a full stop. These sentences are sent to the back end one by one to be processed. The sentence is tagged using Brill's tagger, assigning each word to a particular part of speech. Using this information, rules

are applied and relevant information consisting of which words are entities, attributes, relationships and cardinalities are stored in text files. These text files are then used to generate an ERD using the DeZign tool.
The submit button remains disabled when the user has not entered text, once the user types text into the text field this button is enabled and the user can now submit the problem statement for processing to generate an ERD.

Save:
The problem statement typed in the text field can be stored by the user for further use or reference using this option. The problem statement is saved as either a .txt or .rtf file in the directory in the computer whose location is given by the user.

Exit

To exit the program, the user clicks on this button and the program closes.

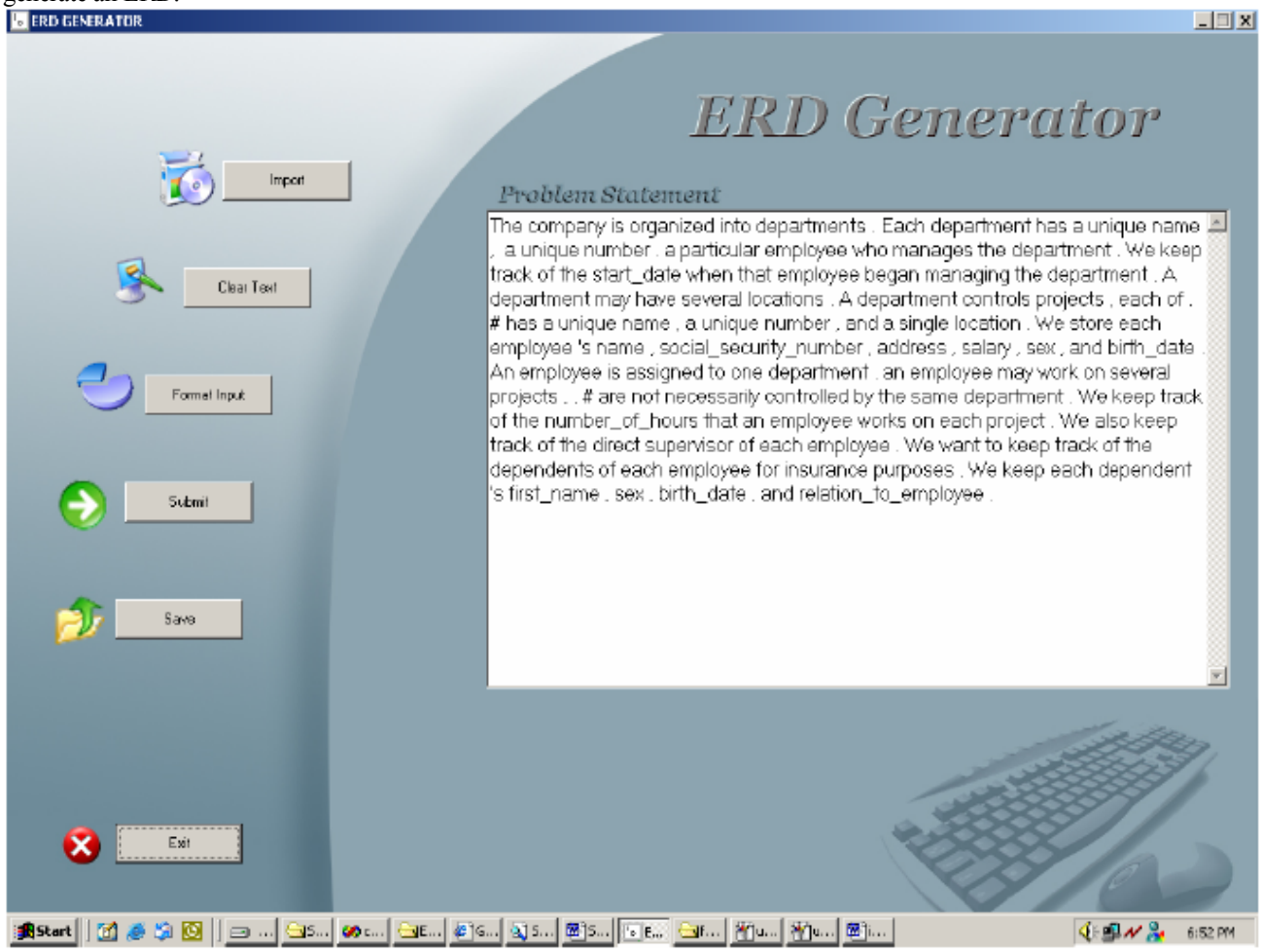

Figure 4.1 - User Interface

Input Assumptions/Constraints

Sentences are entered by the user either directly in the text field or the user has the option to import the problem statement from a text file. A number of limitations have to be put on the user when he types the description. These have been stated below:

1. User input should conform to all rules of English grammar.
2. It is recommended that user should input text in subject-verb-object format.
3. The software does not cater the first person. E.g. we assign a particular id to a department.
4. The words ‘we’, ‘they’, ‘them’, ‘I’, ‘he’, ‘she’ etc. are not allowed. First, second and third person is not allowed.
5. User cannot enter words like number of hours or phone number. These words

should be of the form number_of_hours or phone_number. Words should be entered together as one noun.

6. Questions are not allowed in a scenario
7. Past tense not allowed.
8. Allow the word 'and' only when it terminates a list of attributes. E.g. the department consists of name, id and phone_number. Do not use 'and' otherwise in these sentences break the sentence into two.
9. Cannot use sentences like, e.g. usually each patient.
10. Cannot use semicolons, and other special characters. Commas and full stops are allowed. The system puts a space between the commas and full-stops on its own before running the rules algorithm.

Tagging

"Many corpora are, in addition to structural and bibliographic information, annotated with linguistic knowledge. The most basic and common form this annotation takes is marking up the running words in the corpus with their *part of speech tags*. This adds value to the corpus because, for example, searches can be performed not only on the word-forms as strings but also on whether they belong to a certain linguistic category. Such tags are typically taken to be atomic labels attached to words, denoting the part of speech of the word, together with shallow morph syntactic information, e.g. they specify the word as a proper singular noun, or a plural comparative adjective. For English and other Western European languages, for which most such annotated corpora have been produced, the tag-set size ranges from about forty to several hundred distinct categories.

To label the words in the corpus with their PoS, we fist need a lexicon or morphological analyzer that gives all the possible tags of a given word-form. Part-of-speech taggers then take as their input all these possible morphosyntactic interpretations of the word-form and output the correct interpretation, given the context in which the word-form appears.

There has recently been an increased interest in statistically based part-of-speech taggers, which use the local context of a word form for morphosyntactic disambiguation. Such taggers have the advantage of being fast and can be automatically trained on a pre-tagged corpus. Their success rate depends on many factors, but is usually, for tag-sets of about 100 tags and for Western European languages, at or below 96%. The best known is Brill's rule based tagger**.** In the training phase, this tagger makes an initial hypothesis about the correct tags. In an iterative fashion it then betters its performance with regard to the training corpus by postulating context dependent tag rewrite rules. The advantage of Brill's tagger in comparison with HMM taggers is that the rule-set it generates is more perspicuous than the transition-weight tables of the HMM taggers. Namely, it often turns out to be advantageous to manually correct the automatically induced knowledge of the tagger and it is simpler and more obvious how to change explicit tag rewriting rules than it is changing tables of numbers. Brill's tagger is written in C, with source code and documentation available.

In Brill a trainable rule based tagger is described that achieves performance comparable to that of stochastic taggers. Training this tagger is fully automated but unlike trainable stochastic taggers linguistic information is encoded directly in a set of simple non stochastic rules.

The primary goal of Eric Brill's research is to make information access and the use of computing devices a natural and painless task. As a step towards this goal, he is trying to make computers proficient at processing human language. He has pursued a line of research that falls under the rubric of Empirical Natural Language Processing [2, 3, 4, 5].

EERD Mapping

In many information systems projects, requirements are primarily documented in English, and then database designers convert these English descriptions into database schemas in terms of ERDs. During the course of this project we have proposed a number of rules to generate an ERD diagram from English sentence structure. The basics constructs of English such as noun, verb, adjective, and adverb are found to have counterparts in the ERD. Finally and example is used to demonstrate the applicability of these rules in database design.

Description of Rule

In this section we present rules for translating English sentences into ERD. Although we call them "rules", they might better be viewed as "guidelines", since it is possible to find counterexample to them. The following are the detailed explanations of the translation rules. Some of the sample rules are given below. The list

is huge and can be extended depending on the application and use.

Rule 1

A noun followed by a verb and then a noun forms: Both the nouns form the two entities

There exists one relationship, the verb, between the two entities.

English Statement: Various items are supplied by a supplier.

Analysis and translation: "Items" and "supplier" are nouns, they become the entity and "supplied" becomes the relationship between them. "Items" is changed to "item".

ERD: The corresponding ERD is shown in Figure 4.1.1.

Figure. 4.1.1 - Rule 1

English Statement: A person may own a car. A person may belong to a political party.

Analysis and translation: Note that "person", "car" and "political party" are nouns and therefore correspond to entity types. Note also that "own" and "belongs to" are verbs and therefore correspond to relationship types.

ERD: The corresponding ERD is shown in Figure 4.1.2.

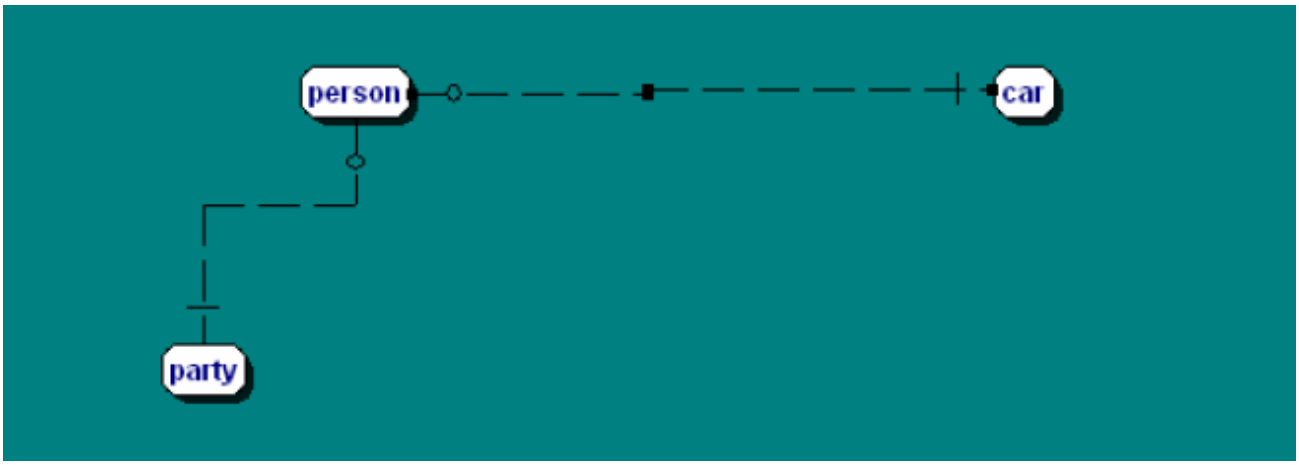

Figure. 4.1.2 - Rule 1

Rule 2

If a noun is followed by has or have and then by noun(s), then: The first noun found is an entity

The second noun(s) found are one or more attributes of the entity.

English Statement: Each department has a unique name and unique number.

Analysis and translation: "Department" is the noun and "name" and "number" are the attributes of department.

ERD: The corresponding ERD is shown in Figure 4.2.

Rule 3

If a noun is found with an apostrophe 's' followed by other noun then:

a) The first noun is an entity.

b) The ones that follow form attribute of the entity.

English Statement: Each employee's email and name is stored.

Analysis and translation: "Employee" is the entity and "email" and "name" are its attributes.

ERD: The corresponding ERD is shown in Figure 4.3

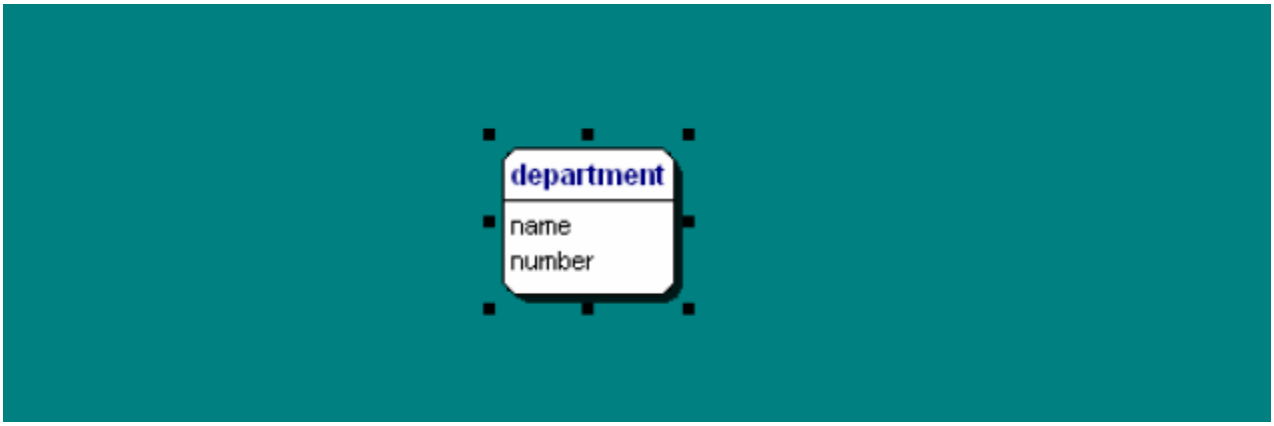

Figure. 4.2 - Rule 2

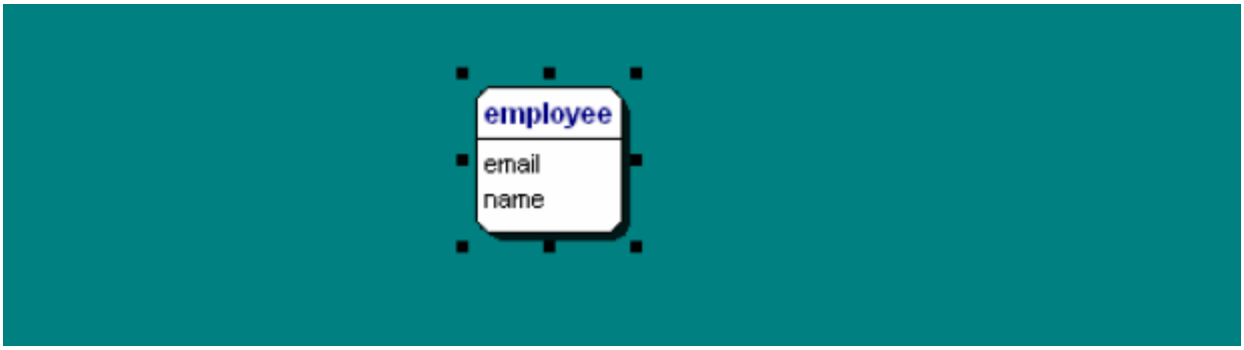

Figure. 4.3 - Rule 3

High-Level Use Cases

The following high level use cases are catered by our tool. These are the ones that are the external actor interacts with.

- Input Problem Statement
- Save Input
- Generate ERD
- Save ERD
- Open ERD
- Export ERD

Analysis and Results

The following calculations were performed for the analysis of the results obtained through the application of above mentioned algorithm.

Text file has been divided into words. Each word contains a tag. This tag is used for the identification of each word i.e. either this word is a noun, proper-noun, verb, adjective etc. Once words have been recognized in a sentence, then algorithm make the sequence of these tagged words. On the basis of this tagged sentence algorithm decides which rule is feasible for this sentence. Now, entities, attributes and relationships have been identified in this sentence. Similarly, this process repeats for each sentence in the text file. As an example, summarized results generated algorithmically in 5 different text files are shown for each entity, attribute and relationship in the Table 1,Table2 and Table 3. The table1 shows the total number of entities manually identified, total number of entities identified by the proposed algorithm, E as O actual entity which were termed as other (attribute or relationship), O as E actual other (attribute or relationship) termed as a particular entity. The table 2 shows the total number of attributes manually identified, total number of attributes identified by the proposed algorithm, A as O actual entity which were termed as other (entity or relationship), O as A actual other (entity or relationship) termed as a particular attribute. The table 3 shows the total number of relationships manually identified, total number of relationships identified by the proposed algorithm, R as O actual entity which were termed as other (attribute or entity), O as R actual other (attribute or entity) termed as a particular entity. Formulas for calculating Recall and Precision values for entities, attributes and relationships are as follows.

$$\text{Entity Recall} = \frac{\text{Entities Identified Correctly}}{\text{Entities Identified Correctly} + E_{asO}} \quad (1)$$

$$\text{Entity Precision} = \frac{\text{Entities Identified Correctly}}{\text{Entities Identified Correctly} + O_{asE}} \quad (2)$$

Precision defines the proportion of the classified words (Entities/Attributes/Relationships) which are actually correct whereas recall depicts the sensitivity, or the proportion of the correct results obtained.

Table 1 Entity Recall & Precision

File	E(manual)	E(algo)	E as O	O as E	E Recall	E Precicion
1	7	6	1	0	75	85.71%
2	10	8	1	2	72.72%	66.66%
3	14	11	3	0	64.70%	78.57%
4	25	23	1	1	88.46%	88.46%
5	17	15	2	0	78.94%	88.23%

$$\text{Attribute Recall} = \frac{\text{Attributes Identified Correctly}}{\text{Attributes Identified Correctly} + A_{asO}}$$

$$\text{Attribute Precision} = \frac{\text{Attributes Identified Correctly}}{\text{Attributes Identified Correctly} + O_{asA}}$$

Table 2 Attribute Recall & Precision

File	A(manual)	A(algo)	A as O	O as A	A Recall	A Precision
1	19	16	2	1	76.19%	80%
2	26	21	4	1	70%	77.77%
3	29	28	1	0	93.33%	96.55%
4	43	39	3	1	84.78%	88.63%
5	33	27	4	2	72.97%	77.14%

$$\text{Relation Recall} = \frac{\text{Relations Identified Correctly}}{\text{Relations Identified Correctly} + R_{asO}}$$

$$\text{Relation Precision} = \frac{\text{Relations Identified Correctly}}{\text{Entities Identified Correctly} + O_{asR}}$$

Table 3 Relation Recall & Precision

File	R(manual)	R(algo)	R as O	O as R	R Recall	R Precision
1	5	3	2	0	42.85%	60%
2	11	8	1	2	66.66%	61.53%
3	13	9	1	0	64.28%	69.23%
4	17	12	2	3	63.15%	60
5	26	20	5	1	64.51%	74.07%

It has been observed form the table 1, table 2 and table 3 that the accuracy level of entities and attributes identification is very good but the identification of relationships among these entities and attributes is below the satisfactory level. Attributes like id, courseid, deptNo and other short names are the major reason to down the accuracy level of attributes identification. These short names also contribute a lot to decrease the level of identification of relationships among the entities. It has been analyzed that if the writer of the text file uses full words rather than the short words than this accuracy level can be increased up to some satisfactory level. The accuracy for each of the Entities, Attributes and Relationships is 79%, 82% and 63% respectively. Average precision for all of the entities, attributes and relationships is 81.5%, 84% and 67% respectively and average recall for all of the entities, attributes and relations is 76%, 79.4% and 60.2%. Accuracy for the whole system is about 75%.

Future Directions:

The point where we export our text file to the external tool namely DeZign leaves a lot of room for future work in this field. The nature of any such future work can be broadly categorized as follows:

- These rules or guidelines presented are not the extendable. New rules can be added and the presented ones can be modified.
- I have proposed certain constraints and asked the user to give the description to the system that fulfills the constraints. Work can be done to pre process the user description before input to the system, such that the textual input is automatically set according to the constraints.
- The use of semantics rather than structural analysis to help infer many such things that have not been catered e.g. cardinalities, weak attributes, composite attributes etc.
- Implementation of integrity constraints.
- Automated generation of tables that are used by Relational Database Management Systems (RDMS) to implement and maintain databases

Abbreviations

Following is a list of the abbreviations that have been used throughout the documentation.

E = Entity
A-> E = Attribute of Entity
A-> R = Attribute of Relationship
R = Relationship
C = Cardinality
RR = Recursive relationship
ERD = Entity Relationship Diagram
EERD = Extended Entity Relationship Diagram

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STYX : A CML based chem.-informatics facility

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Abstract: Developing countries such as Pakistan lose out the benefits of global cheminformatics research unless immediate actions are taken to strengthen the infrastructure for their participation. They rely on dry labs because they don't have the wet labs. They need infrastructure so that cure can be found of the diseases and infections which attack the local human, animal and plant population as international pharmaceutical companies are less interested in these "poor man " diseases for lack of financial turnover. We feel that instead of supplying disease information and data to the foreign companies, we would then use this information to discover new drugs.
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Keywords: STYX; CML based chem.-informatics facility

1. Introduction

The research community has not been able to benefit from the computational power of modern computational machines because of the ad hoc manner in which the data has been recorded over the years [1,2,3]. Only recently, some efforts have been made to mitigate the problems of disparate, heterogeneous, non standardized and distributed data resources. The major impediment to these efforts is the tremendously huge amount of legacy data. We feel that a dependency on these primary biological data resources which are based in the developed countries will result in inheriting these problem s[4,5,6]. Computational power can help Research work but we lack of

- Specialized DBMS for data that constitutes chemical research.
- Semantic access to already done research work.
- Ontological descriptions for already existing research work.
- Interoperability between various data resources.

We are designing a Database through which researchers and scientists will be able to easily retrieve and use the research work done by other scientists, furthermore they will be able to access the data semantically and ontologically [7].

Although there are few databases available in which chemical data is available but it has only developed country data and there is no data available of local diseases of developing countries [8].
Furthermore the data in these databases are in the form of html and xml, but there are not semantic or ontological search methods available in them, so they are not of much use to scientists and researchers.
We will design a database in which chemical data is represented in a standard format CML (chemical markup language), and will make RDF (resource description framework) graphs of such data [9,10]. After making these changes to the available data formats of such research work we will be able to make semantic and ontological search on it and also by representing this data in CML format we will have an advantage that there are some readymade CML enabled applications available, which makes it easy to manipulate and analyze the data[11]. So not only the scientists and researchers can get the data in more appropriate format, in addition if interested they will also be able to extend this work. Furthermore this data would be available worldwide, so other scientists in the world would aware of it and they might be helpful to find the cure of that disease.

2. Semantic Searches for Chemical Biology

Drug discovery is a vast field, and in this fast going era we daily met across some new diseases that originate from different places of the world, and some time scientists get aware of them, when these diseases exist in developed countries, and mostly don't get aware of them when such diseases originate in under developed countries because of those very

good pharmaceutical companies discourage research work to take place in such under developed countries because of negligible profits, and diseases in that area are left untreated unless they don't make their way to such developed countries. For this reason these diseases are called 'Orphan Diseases'[10,11,].

Now how, what we are doing will make good to this entire imbalance which is mentioned above? This will remove the distances between researchers and scientists present in different areas of world, they will be merged in to single unit, and it will be like every new research or experiment results will be available to you anywhere in the world the very next minute it is done. This will make the all the diseases to be cured a global problem because of its being available instantly in useful form and they will start working together in order to cure diseases [2,11].
Although there is research being done in making web searches semantic, but what if we make CHEM informatics semantically searchable? This will be very helpful for scientists to discover cure of diseases being problem of today, and it should not be the case that someone is wasting time in trying to cure what have already been cured somewhere else and giving time to what have to be cured yet, so combining their efforts and strengthening the research work to take it to a whole new level of collaborating their research work.

3. Cheminformatics tools: IT outsource model

As drug discovery gets more technology-focused, many software companies are already functioning as lab less pharmaceutical companies, cashing in on the market for drug discovery informatics (cheminformatics) and bioinformatics. Also, the increase in external licensing deals for drugs by pharmaceutical companies has resulted in more support for smaller, corporate or university drug development research units, and thus a demand for affordable bioinformatics and chemoinformatics tools for these groups, and a corresponding need for education in bioinformatics and chemoinformatics techniques. The financial return for such IT investments is quantifiable and significant. Successful completion of this project will help local IT companies to grow as Technology Driven Companies providing a technological platform to provide various types of goods and services in molecular biology and drug development chain and will try to emulate the development of IT sector international outsourcing. With the bioinformatics market size expected to hit US$ 3 billion by the year 2011 and a phenomenal growth in its offshoots like cheminformatics and pharmacogenomics, we feel that it is the right time for Pakistan to build bioinformatics/cheminformatics infrastructure and capture a large pie of the lucrative market [5.7].

As major pharmaceutical and genome-based biotech companies invest heavily in software, Pakistan's IT companies have a great business opportunity to offer complete solutions to major pharmaceutical and genome-based biotech companies in the world. This project aims to be only a small step in this direction.

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Nurses' related factors influencing the use of physical restraint in critical care units

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Abstract: Although physical restraints are used commonly to maintain the safety of critically ill patient, the use of physical restraints is associated with many adverse effects. Therefore, several attempts have been made to identify and control factors affecting restraint utilization in critical care units (CCUs). This study aims to identify nurses' related factors influencing the use of physical restraints in CCUs. This study was conducted in three of the CCUs of Alexandria Main University Hospital. Fifty critical care nurses, working in the above mentioned settings, who apply physical restraints, were recruited sequentially in this study. Fifty patients who were restrained and assigned to the observed nurses were included in this study. Patients who were restrained for a period of less than two hours were excluded. Two tools were used to collect the required data; nurses' restraint performance checklist, and nurses' related factors influencing the use of physical restraint questionnaire. It can be concluded from this study that older nurses and those with higher qualification and years of experience have better restraint related performance than others. Nurses' knowledge and performance are in need for improvement. Therefore, it is recommended to conduct in-service training programs for nurses working in CCUs on restraint utilization and restraint alternatives. The hospital should develop evidence based guideline on physical restraining to be available for all nurses and physicians in order to follow.

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Keywords: Nurse; factor; physical restraint; critical care unit

1. Introduction:

Critically ill patients are characterized by the presence of actual and/or potential life-threatening health problems, requiring continuous observation, intervention and an extraordinary dependence on the health care providers and possibly technology [(1-3)]. Maintaining an optimal level of patient's comfort and safety while providing monitoring and treatment to critically ill is essential [(4)]. Agitation is a major problem for critically ill patients in intensive care units (ICUs). Agitation is not a diagnosis but, rather, a symptom of many diseases and disorders. It might result from pain, adverse effects of medications or fluid and electrolyte imbalance, environmental or psychosocial stressors, or psychiatric syndromes. Physical restraints have been used to ensure the safety of agitated patients through preventing their interference with their treatment, limiting physical hyperactivity, facilitating necessary care, and avoiding harming themselves or others. However, the use of physical restraints is a controversial issue and may present nurses with practical, legal, and ethical dilemmas [(5)].

In relation to the practical dilemmas driven from the restraint use, two broad categories of restraints related injuries are reported in a number of studies; direct and indirect injuries. Direct injuries involve physical injuries caused by external pressure from the restraining device, including; lacerations, bruising, and/or strangulation. On the other side, indirect injuries include adverse outcomes related to the enforced immobility of a person, and included increased mortality rate, development of pressure sores, falls, or failure to be discharged home [(2,6,7)]. Regarding ethical and legal dilemmas related to restraints' use, critical care nurses are usually faced by these types of dilemmas when restraining a patient unless there is an appropriate and adequate clinical justification and that prior alternative interventions have been tried and deemed unsuccessful. A number of researchers indicated that it is unacceptable and unethical to use restraints because of low staffing levels or to control disruptive patient's behaviours and they indicated that if nurses are caring enough to decide what is best for their patients, they should take into account the needs and desires of patients, and consequently the patient's autonomy [(8,9)].

Given the evidence of practical, legal, and ethical dilemmas associated with the restraint use, critical care nurses continue to use physical restraints in ICUs. Studies have shown that decisions to use physical restraints and their application are affected by a number of factors such as; nurses' number,

qualification, experience, and knowledge regarding restraint use [5].

In order to understand why nurses choose to use physical restraints, and what are the most common malpractices associated with their use, nurses' related factors influencing their decision to initiate a restrain and their practices while applying or maintaining physical restraining have to be examined. Once factors and malpractices are identified, strategies can be made toward finding safe alternative choices for restraining and corrective measures for malpractices, which are more consistent with professional practice and quality care. Thus, this study was conducted to identify nurses' related factors influencing the use of physical restraints in CCUs.

2. Material and Methods

Research design: descriptive study design.

Study aim: Identify nurses' related factors influencing the use of physical restraints in CCUs

Research questions: What are nurses' related factors influencing the use of physical restraints in CCUs?

Materials

Setting:

This study was conducted in three of the CCUs of Alexandria Main University Hospital, namely; the Casualty Intensive Care Unit (Unit I), the General Intensive Care Unit (Unit III), and the Recovery Care Unit.

Subjects:

Fifty critical care nurses, working in the above mentioned settings, who apply physical restraints, were recruited sequentially in this study. Intern nurses were excluded from the study sample. Fifty patients who were restrained and assigned to the observed nurses were included in this study. Patients who were restrained for a period less than two hours were excluded.

Tools:

Two tools were used to collect the required data; nurses' restraint performance checklist, and nurses' related factors influencing the use of physical restraint questionnaire

Tool (I): Nurses' restraint performance observational checklist:

This tool was adapted from **Potter *et al.*** [10]. It is used to assess nursing performance while applying and providing maintenance care of physical restraint. It consists of three parts. Part "1" includes type of the ICU, the shift (morning, evening or night) when the observation was taken, and nurse to patient ratio. Part "2" involves restrained patients' related characteristics, such as; age, medical diagnosis, and past history. Part "3" was used to observe nurses' practices while applying and maintaining the physical restraint. It contains five main sections covering the main steps of restraint use and care; assessment, preparation, application, post care and maintenance, and documentation. First section is for assessment, which involves items to be assessed before the application of a physical restraint, such as; indication of applying restraint, physician's order, and the site of restraint. Second section is concerned with the preparation for restraining which involves; preparation of equipment, patient, and environment. Third section is for the application of the physical restraint including practices such as; padding bony prominences, and securing the restraint accurately. Section four involves post care practices such as; washing hands, and performing regular care while the restraint is maintained. Finally, section five is for documentation, which includes items such as; documentation of the type, location, time, indications, and unexpected outcomes for restraining.

The total score for this observational performance checklist is 30 points, in which; each practice performed completely and accurately is graded as one point. Incorrect, incomplete or not done practice is graded as zero. The maximum possible score for the physical restraint check list is 30. The cut point for "Good" is greater than 80% of total scores, "Moderate" is between 60% to 80% of total scores and "Poor" is less than 60% of total scores.

Tool (II): Nurses' related factors influencing the use of physical restraint questionnaire:

It was developed by the researchers after reviewing the related literature [1-4,10] and is used to identify nurses' related factors influencing the use of physical restraints in CCUs. It includes four parts. Part 1 is for nurses' characteristics, such as; age, length of clinical experience, qualification, and level of education. Part 2 assesses nurses' knowledge regarding physical restraint in general, such as; indications for application and removal of restraint, and complications of physical restraining. Part 3 assess nurses' knowledge concerning physical restraining related practices; including; knowledge regarding assessment, preparation, application, post care and maintenance, and documentation of restraining. Eventually, part 4 assesses nurses' knowledge regarding legal and ethical factors related to the use of physical restraint.

For parts 2-4, the answer for each question is scored by the same method, as in tool I. The correct response is scored as "1" and incorrect response or do not know as "0". The maximum possible score for the questionnaire was 45. Also, the cut point for "Good" is greater than 80% of total scores, "Moderate" is between 60%to 80% of total scores, and "Poor" is less than 60% of total scores.

Methods:

Permission was obtained from the hospital administrative authority to collect the necessary data. Validation of the study tools was assessed by presenting them to five experts from the critical care nursing field. A pilot study was carried out on 5 nurses and their assigned patients who were restrained by those nurses to evaluate the clarity and applicability of the study tools. They were excluded from the total sample, and the necessary modifications were done.

The Kuder-Richardson reliability coefficient of the questionnaire was 0.63 for all items. An informed consent was obtained from each nurse included in the study. The anonymity and confidentiality of responses, voluntary participation and right to refuse to participate in the study were emphasized. The researcher explained to the nurses the objectives of the study orally, additionally to the written explanations on the covering letter of the questionnaire.

Data collection: Data was collected over the period from 15/1/2009 to 30/4/ 2009. Nurses' restraint performance observational checklist (tool I) was used to assess the performance of each nurse while applying physical restraint and providing its maintenance care. The researchers observed each nurse during applying physical restraints over different three shifts. Nurses' related factors influencing the use of physical restraint questionnaire (Tool II) was distributed to the nurses, who applied physical restraints and providing their maintenance care to identify nurses' related factors affecting the utilization of physical restraint. Nurses were asked to answer the questionnaire and to bring it back to the researchers (at the end of the shift). The questionnaire completion time was about 15 minutes.

Statistical Analysis: Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) version 13.0. Results were illustrated in cross tabulation. Descriptive statistics (frequencies, percentages, means and standard deviations) were used to describe the study sample. F test was used to compare means of groups within the same sample. Pearson correlation coefficient was used to assess association between nurses' performance and their knowledge. Statistical significance was set at p value <0.05.

3. Results:

Table (1): presents personal characteristics of the studied nurses. It is found that 58.0% of the nurses have a bachelor degree, 22.0% are graduated from the technical nursing institute, and only 20.0% are graduated from the secondary nursing school. As for nurses' years of experience at the CCUs, about two thirds of the nurses (66.0 %) have an experience of less than 5 years. Regarding nurses age, it is found that 26.0% are aged below 20 years, and 56.0% are between 20-29 years.

Table 1: Personal characteristics of the studied nurses at the critical care units:

Characteristics	Nurses (n=50)	
	No.	%
Nurses' qualification		
B.Sc. Nurses	29	58.0
Technical Institute of Nursing	11	22.0
Diploma secondary nursing School	10	20.0
Total	**50**	**100.0**
Years of experience at the CCUs		
< 5 years of experience	33	66.0
5 – 10 years of experience	10	20.0
10 + years of experience	7	14.0
Total	**50**	**100.0**
Mean ± S.D.	4.16±3.803	
Minimum	1	
Maximum	15	
Age (Years)		
< 20	13	26.0
20-29	28	56.0
30-39	9	18.0
Total	**50**	**100.0**

Table (2): Shows characteristics of the restrained patients. It is found that patients' ages are ranged between 15 and 85 years old; most of them are more than 45 years old (86%), with a mean of 59.24± 16.400 years. The study includes 35 males (70%) and 15 females (30%) patients. Regarding the medical diagnosis, 21 (42%) patients has respiratory disorders and 14(28%) had Neurological disorders.

Table 2: Characteristics of the restrained patients

Characteristics	Restrained patents (n=50)	
	No.	%
Age (Years)		
15-44	7	14.0
45-75	32	64.0
>75	11	22.0
Total	**50**	**100.0**
Mean age (Years) ± S.D.	59.24± 16.400	
Minimum	16 years	
Maximum	85 years	
Gender		
Male	35	70.0
Female	15	30.0
Total	**50**	**100.0**
Diagnosis		
Trauma	3	6.0
Metabolic disorder	2	4.0
Endocrine disorder	2	4.0
Renal disorder	2	4.0
Cardiac disorder	6	12.0
Respiratory disorder	21	42.0
Neurological disorder	14	28.0
Total	**50**	**100.0**

Table (3) displays distribution of the restrained patients according to time shifts, and nurses to patients' ratio. It demonstrates that 13 patients (26%) are restrained in the morning shift, 18 (36%) in the evening shift, while 19 (38%) of them were restrained in the night shift. In relation to the nurse: patient ratio, it is found that 13 (26%) of the patients are restrained when nurse patient ratio is one to one, and percentage of all restrained patient in the unit is 20%, 35(70%) were restrained when nurse patient ratio was one to two, and percentage of all restrained patient is 32%, 2(4%) of them are restrained when nurse patient ratio is one to three, and percentage of all restrained patient was 50%. This means that the percentage of restrained patient increases with increasing nurse to patient ratio.

Table3: Distribution of the restrained patients according to time shifts, and nursing staff to their ratio.

Characteristics	Restrained patients included in the study n = 50		Percentage of all restrained patient in the unit
	No.	%	
Shift			
Morning	13	26.0	
Evening	18	36.0	
Night	19	38.0	
Total	**50**	**100.0**	
Nurse to patient ratio			
One nurse to one patient	13	26.0	20%
One nurse to two patients	35	70.0	32%
One nurse to three patients	2	4.0	50%
Total	**50**	**100.0**	

Table (4) represents nurses' performance and knowledge regarding physical restraints. Regarding nurses performance, it is found that 45 (90%) nurses have moderate performance, 3 (6%) have good performance, and 2

(4%) of them have poor performance. Concerning their knowledge, it is found that 32 (64%) of nurses had moderate knowledge, 14 (28%) has poor knowledge, while, only 4 (8%) of them had good knowledge.

Table 4: Nurses' performance and knowledge regarding physical restraints:

Rating	Frequency (n=50)	%
Performance		
Poor	2	4.0
Moderate	45	90.0
Good	3	6.0
Total	**50**	**100.0**
Knowledge		
Poor	14	28.0
Moderate	32	64.0
Good	4	8.0
Total	**50**	**100.0**

Table (5) shows the relationship between nurses' restraint related performance and their qualification. It presents that there are statistical significant relationships between all phases of nurses' performance, except for the preparation phase, and with the exclusion of the documentation because none of nurses document anything. It was found that increasing the performance score is associated with higher nurses' qualification related to restraining. p values for the assessment, procedure, post care phases of performance are 0.005, 0.038, and 0.001, respectively. Also, there is a statistical significant relationship between total performance score and qualification, where (F = 10.669, p = 0.000), in which mean performance scores seem better with higher qualifications

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Table 5: The relationship between nurses'restraintrelated performance and their qualification

Physical restraint practices	Critical care units			Performance score (Mean ± SD)	F test
	Nurses' qualification	No.&%			
		No.	%		
Assessment	B.Sc. Nurses	29	58.0	4.00±0.000	F = 5.866, p = 0.005*
	Technical Institute of Nursing	11	22.0	4.00±0.000	
	Diploma (Secondary nursing School)	10	20.0	3.64±0.674	
	Total	**50**	**100.0**	**3.92±0.340**	
Preparation	B.Sc. Nurses	29	58.0	2.50±0.527	F = 0.018, p = 0.982
	Technical Institute of Nursing	11	22.0	2.48±0.574	
	Diploma (Secondary nursing School)	10	20.0	2.45±0.522	
	Total	**50**	**100.0**	**2.48±0.544**	
Procedure	B.Sc. Nurses	29	58.0	5.00±0.00	F = 3.523, p = 0.038*
	Technical Institute of nursing	11	22.0	4.90±0.310	
	Diploma (Secondary nursing School)	10	20.0	4.64±0.505	
	Total	**50**	**100.0**	**4.86±0.351**	
Post care	B.Sc. Nurses	29	58.0	9.70±1.059	F = 8.499, p = .0.001*
	Technical Institute of Nursing	11	22.0	7.64±2.920	
	Diploma (Secondary nursing School)	10	20.0	9.62±1.083	
	Total	**50**	**100.0**	**9.20±1.629**	
Documentation	B.Sc. Nurses	29	58.0	0.00±0.000	-
	Technical Institute of Nursing	11	22.0	0.00±0.000	
	Diploma (Secondary nursing) School)	10	20.0	0.00±0.000	
	Total	**50**	**100.0**	**0.00±0.000**	
Total	B.Sc. Nurses	29	58.0	21.70±1.567	F = 10.669, p = 0.000*
	Technical Institute of Nursing	11	22.0	21.48±1.526	
	Diploma (Secondary nursing School)	10	20.0	18.55±2.876	
	Total	**50**	**100.0**	**20.88±2.246**	

F: Fisher's Exact Test *p value <0.05.

Table (6): The relationship between nurses' restraint related performance and their years of experience. Regarding total score there is statistically significant relationship between nurse's performance and their years of experience, in which increasing years of experience is associated with an improvement with nurses performance, where (F = 4.045, p = 0.024).

Table (7): shows the relationship between nurses' restraint related performance and their age. Regarding total score of performance, there is statistically significant relationship between nurse's performance and their age, in which increasing age is associated with an improvement in nursing performance, (F = 5.246, p = 0.009).

Table (8): demonstrates Nurses' knowledge concerning restraining performance and their observed performance score. Regarding the assessment of physical restraints, it is found that, 50 nurses (100%) know how to assess the need for restraint and they do it. In relation to checking physician's order, no one 0 (0.00%) perform, 29 (58%) recognize that they have to do. Regarding the procedure, most of nurses 49(98%) who apply restraint, 36(72%) of them know how to apply restraints. For making sure it is not over an IV line or other device 49(98%) performed, 11(22%) know. Regarding attaching the restraint to bed frame, not side rails, all of them 50(100%) perform, 36(72%) know. According to Secure restraints with a quick release site 48(96%) performed, while 12(24%) know.

Regarding post care, assessment of proper placement of restraint and condition of patient's restrained body part at least every 30m minutes, only 33(66%) performed, although 40(80%) of them were know. Remove restraints for 30 minutes every 2 hours, 34(68%) performed, and 9(18%) of them know. In relation to documentation all of them 50(100%) do not perform, 44(88%) but they know.

Table (9): shows nurses' knowledge score concerning restraining performance and their observed performance score. There is no statistical significant relationship is found (r = 0.227, p = 0.112), in which the mean of the performance score = 6.56±1.013, and for knowledge score = 8.86±2.222..

Table 6:The relationship between nurses' restraint related performance and their years of experience

Physical restraint practices	**Critical care units**			**Performance score (Mean ± SD)**	**F test**
	Nurses' experience	**No.&%**			
		No.	**%**		
Assessment	< 5 years of experience	35	70.0	3.89±0.404	F = 0.581, p = 0.563
	5 – 10 years of experience	10	20.0	4.00±0.000	
	10 + years of experience	5	10.0	4.00±0.00	
	Total	**50**	**100.0**	**3.92±0.340**	
Preparation	< 5 years of experience	35	70.0	2.43±0.558	F = 0.512, p = 0.603
	5 – 10 years of experience	10	20.0	2.60±0.516	
	10 + years of experience	5	10.0	2.60±0.548	
	Total	**50**	**100.0**	**2.48±0.544**	
Procedure	< 5 years of experience	35	70.0	4.83±0.382	F = 0.595, p = 0.556
	5 – 10 years of experience	10	20.0	4.90±0.316	
	10 + years of experience	5	10.0	5.00±0.000	
	Total	**50**	**100.0**	**4.86±0.350**	
Post care	< 5 years of experience	35	70.0	8.91±1.738	F = 2.034, p = 0.142
	5 – 10 years of experience	10	20.0	9.70±1.160	
	10 + years of experience	5	10.0	10.20±1.095	
	Total	**50**	**100.0**	**9.20±1.629**	
Documentation	< 5 years of experience	35	70.0	0.00±0.000	-
	5 – 10 years of experience	10	20.0	0.00±0.000	
	10 + years of experience	5	10.0	0.00±0.000	
	Total	**50**	**100.0**	**0.00±0.000**	
Total	< 5 years of experience	35	70.0	20.14±2.522	F = 4.045, p = 0.024*
	5 – 10 years of experience	10	20.0	21.70±1.494	
	10 + years of experience	5	10.0	22.80±1.789	
	Total	**50**	**100.0**	**20.72±2.441**	

F: Fisher's Exact Test *p value <0.05.

Table 7: The relationship between nurses' restraint related performance and their age.

Physical restraint practices	Critical care units			Performance score (Mean ± SD)	F test
	Nurses' age (Years)	No.&%			
		No.	%		
Assessment	< 20	13	26.0	3.69±0.630	F = 4.488, p = 0.016*
	20-29	28	56.0	4.00±0.000	
	30-39	9	18.0	4.00±0.000	
	Total	**50**	**100.0**	**3.92±0.340**	
Preparation	< 20	13	26.0	2.46±0.519	F = 0.44, p = 0.957
	20-29	28	56.0	2.50±0.577	
	30-39	9	18.0	2.44±0.527	
	Total	**50**	**100.0**	**2.48±0.544**	
Procedure	< 20	13	26.0	4.69±0.480	F = 2.468, p = 0.096
	20-29	28	56.0	4.89±0.315	
	30-39	9	18.0	5.00±0.000	
	Total	**50**	**100.0**	**4.86±0.350**	
Post care	< 20	13	26.0	8.00±2.309	F = 5.759, p = 0.006*
	20-29	28	56.0	9.57±1.136	
	30-39	9	18.0	9.78±0.833	
	Total	**50**	**100.0**	**9.20±1.629**	
Documentation	< 20	13	26.0	0.00±0.000	-
	20-29	28	56.0	0.00±0.000	
	30-39	9	18.0	0.00±0.000	
	Total	**50**	**100.0**	**0.00±0.000**	
Total	< 20	13	26.0	19.08±3.040	F = 5.246, p = 0.009*
	20-29	28	56.0	21.07±2.071	
	30-39	9	18.0	22.00±1.225	
	Total	**50**	**100.0**	**20.72±2.441**	

F: Fisher's Exact Test *p value <0.05.

Table 8: Nurses' knowledge concerning restraining performance and their observed performance score.

Selected nurses' practice	Nurses who performed n=50		Nurses who Knew n=50	
	No.	%	No.	%
Assessment of physical restraints				
• Assess the need for restraints.	50	100.0	50	100.0
• Check physician's order	0	0.0	29	58.0
Procedure				
• Apply restraint	49	98.0	36	72.0
• Making sure it is not over an IV line or other device	48	96.0	11	22.0
• Attach restraints to bed frame , not side rails	50	100.0	36	72.0
• Secure restraints with a quick release site	48	96.0	12	24.0
Post Care				
• Assess proper placement of restraint and condition of patient's restrained body part at least every 30m minutes	33	66.0	40	80.0
• Remove restraints for 30 minutes every 2 hours.	34	68.0	9	18.0
Documentation				
• Type and location of restraint.	0	0.0	44	88.0
• Time and reason for application.	0	0.0	44	88.0
• Condition of the skin under the restraint	0	0.0	44	88.0
• Need for continued use.	0	0.0	44	88.0
• Causes of removal restraint.	0	0.0	44	88.0

Table (9): Nurses' knowledge score concerning restraining performance and their observed performance score

Scores	Median	Mode	Range	Mean ± SD	Pearson correlation coefficient (r)
Performance scores	7.00	7	3	6.56±1.013	
Knowledge scores	9.00	10	10	8.86±2.222	r = 0.227, p = 0.112

r: correlation coefficient *p value <0.05.

4. Discussion:

Although physical restraint is used as a safety measure to prevent patients' fall, its use is usually associated with many adverse effects [(11,12)]. In addition, it raises many ethical and practical concerns. Therefore, several attempts have been made to reduce the number of restraints in clinical practice and encourage the use of their alternatives. One of these attempts is to control factors affecting nurses' use of physical restraints [(13,14)]. Therefore, this study was conducted to identify nurses' related factors influencing the use of physical restraints in CCUs.

Although the focus of this study is on nurses' related factors influencing restraint utilization, study findings shows that there are other factors related to patients' characteristics influence restraint utilization. Regarding ***patients' characteristics***, it was found that most of the restrained patients were aged between 45-75 years old, and the mean age was 59 years old. This finding is supported by **Martin** [(15)] who found that advanced *age* is strongly associated with the use of physical restraints. This may be because aging is one of the main factors causing patients' agitation and consequently putting them into the risk of pulling the life support devices and catheters or harming themselves and others [(16)].

For the *medical diagnosis*, this study showed that about half of the restrained patient had respiratory disorders. This may be because critically ill patients with respiratory disorders are always in need for monitoring and supportive respiratory devices and/or tubes. Monitoring and supportive respiratory devices and tubes may range from simple devices, such as; simple masks to the more sophisticated devices, including; mechanical ventilators which most commonly need intubation, nebulizers, or arterial catheters. Therefore, the main reason for restraining these patients may be maintaining and preventing the removal of supportive respiratory devices. This was also the case in other studies conducted in ICUs which found that the most common reason for restraint application reported by nurses was to prevent the critically ill patient from removing medical monitoring or supportive devices[(17,18)].

The current study shows also that about third of the restrained patients had neurological disorders. This may be attributed to the altered level of consciousness and/or the abnormal behaviours associated with their neurological disorders. In this case, without restraining these patients, they will be more susceptible to harming themselves or others and/or pulling out any monitoring or supportive devices or catheters. These findings are supported by **Emerson**[(19)] study which indicated that over half of restrained patients had challenging behaviours and intellectual disabilities; consequently, they were more exposed to the application of restraint.

Regarding ***nurses' characteristics***, this section will present a description of the nurses' characteristics working as factors affecting restraining including; nurses' age, years of experience, qualification, staff level (nurse: patient ratio), and nurses' knowledge. In relation to the *staff level* (nurse: patient ratio), results of this study reveal that the number of restrained patients increased with the decrease in the number of nurses, and this usually occurs in the evening and night shifts more than the morning shift. This may be interpreted as there is a shortage of nursing staff in the evening and night shifts which increase nurses' work overload, consequently advance them to use restraint. The present study finding is in line with **Engberg *et al.* and Martin,** studies [(15,20)]. They indicated that the restraint initiation may be much higher at night, when staffing levels are lower. They indicated that staffing patterns have been cited as a factor that may influence the use of physical restraint. This result is supported also by **Magee *et al.*** [(21)] study which found that the restraint use is inversely proportional to the number of the nursing staff. However, they found that fewer restraints were used on Sundays when there was fewer staff on duty. They postulated their findings that more restraints were used during Sundays and some day shifts because more staff was available to get patients out of bed and so, these patients were then restrained in chairs.

In relation to *nurses' qualification*, the present study found that nurses' performance in applying and maintaining restraining increases with the increase in nurses' qualification. This can be explained by the fact that B.Sc. nurses received some training on restraining while they were

undergraduates as a procedure included in the nursing fundamental course. Nurses graduated from the technical institute of nursing received also training on restraining, although it is brief. While, nurses graduated from the secondary nursing school did not receive any classes or clinical training on physical restraining. In relation to *nurses' experience*, it was found that there is a significant relationship between nurses' performance and nurses' experience. This study showed that nurses with a higher experience are performing the procedure of restraining better than others. These findings are congruent with **Gillis,**[(22)] who argued that day to day activities enhance nurses' experience and improve their performance while applying and maintaining restraining.

The present study revealed there was a significant relationship between nurse's performance and their *ages*, in which, it was found that the level of performance of nurses concerning physical restraints increased with older nurses. These findings are generally in line with **McMillan's** study[(23)] which concluded that professionals mature age-wise nurses who have experience tend to make a better adjustment when compared with younger peers.

The present study demonstrated that *nurses' general knowledge* regarding the practices of applying and maintaining physical restraining as well as their performance were moderate. This could be explained by the lack of training for nurses on physical restraining, the lack of written policies and procedures in ICUs guiding physical restraining and inadequate supervision and guidance by the nurse supervisors. These results are supported by **Karlsson,**[(24)] who indicated that the nursing staff knowledge and attitude regarding the use of physical restraints were strongly associated with their use in practice.

In addition, nurses' performance and knowledge specifically, concerning this performance score are generally moderate and inadequate. It is clear that such low standard of performance in physical restraints practice, is due to a combination of factors, some are related to the hospital and its system, patients and the others are related to nurses themselves. For example, there is no physician order; the nurse can follow which is one of the important legal aspects. This finding may be attributed to lack of cooperation between nurse and physician or lack of physicians' knowledge regarding their role in participating in the decision of restraining a patient. In addition, nurses do not take the patient's consent to apply physical restraint. Also there was no explanation for the patient when applying physical restraints. What is obvious as well was that the absence of any nursing documentations related to restraining. This may be attributed to their belief that restraining procedure is not ethically accepted, so, they do not document any data related to this procedure. Moreover, they may not consider restraining as an important procedure that requires documentation. Current study is in line with a **Korean** study which showed that nurse's records in a patient's chart rarely mentioned the restraint use [(25)].

5. Conclusion and recommendations:

In conclusion, this study reveals that there are a number of factors affecting restraint utilization in CCUs. Factors related to restrained patients include, age, and diagnosis. Elderly patients and patients with respiratory or neurological disorders usually need restraints more than others. In relation to nurses' related characteristics, it can be concluded that those older nurses and those with higher qualification and years of experience have better performance than others. Nurses' knowledge and performance are in need for improvement. Therefore, it is recommended to conduct in-service training programs for nurses working in CCUs on restraint use and its alternatives. The hospital should develop evidence based guideline on physical restraining to be available for all nurses and physicians in order to follow. Further studies have to be conducted identifying other factors that may influence restraint utilization. In addition, Identifying, developing, and testing alternatives to physical restraining is recommended to be the focus of future studies.

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Tracheal suctioning with versus without saline instillation

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Abstract: The primary goal of tracheal suctioning to maintain a patent airway. It is considered as one of the most common procedure in critical care areas. Normal saline instillation into an artificial airway prior to suctioning is utilised by many health practitioners. However, there are conflicting views about its safety. This study was conducted in two phases. Phase "1" aims to determine how often normal saline is used during tracheal suctioning, and determine nurses and physicians' knowledge regarding advantages and disadvantages of normal saline instillation (INS) before suctioning. Phase "2" aims to compare between the effects of suctioning with saline versus suctioning without saline on a number of physiological response parameters. This study was conducted at Causality Care Unit, and General Intensive Care Unit (ICU), Main University Hospital, University of Alexandria; including;. Ninety two nurses and 16 physicians working in the previously mentioned settings were included in the first phase of the study, while 26 adult critically ill patients were included in the second phase of the study. Two tools were used to collect required data; tool (I) tracheal suctioning questionnaire used to collect data for phase "1" and tool (II) physiological response parameters assessment sheet used to collect data for phase "2". Based on the findings of this study, it can be concluded that nurses and physicians frequently use saline before suctioning. A considerable number of them recognize the most common advantages and disadvantages to the INS. In relation to the comparison between suctioning with and without INS, this study shows that INS carries out several risks, including; significant elevation in $PaCO_2$ immediately after suctioning and reduction in oxygen tension and saturation, 5 minutes after suctioning. So, nurses and physicians have to be aware on these disadvantages of INS. In addition, alternative measures facilitating liquefying secretion and its removal have to be utilized instead of INS.
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Key words: instillation of normal saline, suction, physiologic response to suction.

1. Introduction:

Tracheal suctioning (TS) is an essential and frequently performed procedure for patients requiring intubation and mechanical ventilation; in which patients may need to be suctioned between 3 and 24 times or more a day [(1)]. By TS, secretions from the tracheobronchial tree are cleared, guaranteeing optimal oxygenation and avoiding accumulation of secretions, leading to tube occlusion, increased work of breathing, atelectasis, and pulmonary infections [(2)]. Critically ill patients are highly dependent on collaborative skilled health care members throughout all aspects of their care. Nurses and physicians make up the largest group of health care members and their collaboration in making decisions significantly affects patients' outcomes. Although suctioning procedure is considered as one of the nursing tasks, physicians participate in taking decisions related to its techniques and safety. One of the challenges facing the critical care nurse and the physician, when performing tracheal suctioning, is ensuring that the procedure is performed effectively without any harm. Normal saline has been widely utilized with tracheal suctioning. Clinicians use normal saline believing it lubricates the suction catheter, enhances a cough, breaks up pulmonary secretions and helps with their removal during suctioning, especially in the presence of thick secretions [(3)].

Although the INS during TS has been used for years, evidence shows that such instillation is controversial. A study finding indicates that mucus and water are like oil and water, cannot be mixed in vitro, even after vigorous shaking [(4,5)]. In addition, after instillation of saline, extra breaths are given with manual resuscitation bag (MRB) or via the ventilator, or both. In each of these circumstances, the flow of air into the lung is accelerated. If any mucus is loosen via instillation, this rapid flow of air most likely transports these secretions farther down the bronchial tree, making the secretions more difficult or impossible to retrieve with a suction catheter. The unretrieved fluid could interfere with alveolocapillary oxygen exchange, resulting in declines in oxygenation. Furthermore, INS stimulates patient to cough forcefully which may increase mean arterial pressure (MAP) and intracranial pressure [(3,6,7)]. Regarding risk of infection, it was found that INS causes dislodgment of bacterial colonies up to 5 times when normal saline is instilled, and therefore this

practice may contribute significantly to lower airway contamination [8].

On the other hand, other researchers have shown conflicting results. Two studies compared the oxygenation effects of suctioning with and without normal saline resulted in the absence of a statistical significant effects on oxygen saturation [9,10]. **Choi and Jones** [11] study suggested that manual hyperinflation associated with the instillation of 1 ml normal saline in conjunction with suction induces beneficial changes in respiratory mechanics in mechanically ventilated patients. Another study investigated the effect of INS on respiratory mechanics demonstrated no statistically significant difference in pulmonary compliance in control or intervention groups [12]. **Caruso and colleagues** [13] and **Reeve** [14] demonstrated that INS reduces the incidence of ventilator associated pneumonia in intubated patients.

Lack of researches carried out on adult patients comparing between using or not saline during suctioning, in addition to the conduction of a recent systemic review [15] reopened the debate again. The systematic review was conducted to investigate the efficacy and safety of the INS prior to suction. Its results reflect the poor quality of the available articles on examining the effect of INS prior to suction. The conclusion of this systemic review emphasized that there is little evidence of benefit but also minimal evidence of safety risks and recommended the induction of controlled trials of better quality and more clinically relevant outcomes before this technique is either accepted or rejected. Despite conflicting results on INS, this manoeuvre is being used frequently in practice, and sometimes incorporated into written guidelines. Therefore this study was conducted to determine how often normal saline is used during tracheal suctioning and nurses' knowledge regarding advantages and disadvantages of this manoeuvre. In addition, the current study will compare between the effects of suctioning with versus without saline on a number of physiological stress response parameters, including; hemodynamic parameters, oxygenation parameters, cough response, and lung mechanics.

2. Material and Methods:

Research design: This study was conducted in two phases;

Phase "1": Descriptive design.

Phase "2": Single case repeated measures quasi-experimental design, in which each patient was utilized as his/her own control. Intervention was suctioning with saline, while the control was suctioning without saline.

Aims: Phase "1":

- Determine how often normal saline is used during tracheal suctioning.
- Determine nurses and physicians' knowledge regarding advantages and disadvantages of INS before suctioning.

Phase "2":

- Compare between the effect of suctioning with saline versus suctioning without saline on physiological response parameters, including; hemodynamic parameters (hear rate "HR", systolic blood pressure "SBP", diastolic blood pressure "DBP", and Mean arterial pressure "MAP"), oxygenation parameters (pH, $PaCO_2$, PaO_2, HCO_3^-, and SaO_2), cough response, and lung mechanics (respiratory rate "RR", and dynamic compliance "Cdyn").

Research Hypotheses:

Phase "1":

- Normal saline is always used during tracheal suctioning
- Nurses and physicians cannot determine advantages and disadvantages of suctioning with INS

Phase "2":

- No statistical significant difference will be found between the effect of suctioning with saline and suctioning without saline on physiological response parameters; hemodynamic parameters (HR, SBP, DBP, and MAP), oxygenation parameters (pH, $PaCO_2$, PaO_2, HCO_3^-, and SaO_2,), cough response, and lung mechanics (RR, and Cdyn).

Settings: This study was conducted at two of the intensive care units (ICUs) of the Main university Hospital, University of Alexandria, Egypt, including; Causality Care Unit (Unit I), and General ICU (Unit III).

Subjects: for phase "1": 92 nurses and 16 physicians who were working in the previously mentioned settings and accepted to participate in the research over the period from 1/4/ 2010 to 15/4/ 2010 were included in this phase. *For phase "2":* 26 adult critically ill patients of both sexes were recruited sequentially in this study. All of patients were receiving mechanical ventilatory support via a tracheal tube either endotracheal or tracheostomy tube. Patients excluded from the study were those with refractory hypoxemia because they may not show any changes in oxygenation parameters [12] and those who are on muscle relaxants. This research

phase was conducted over the period from 30/4/ 2010 to 30/9/ 2010

Tools:

Two tools were used to collect the required data; tool (I) for phase "1" of the study, and tool (II) for phase "2".

Tool (I) tracheal suctioning questionnaire: this questionnaire was adopted from **Schwenker *et al.*, [16].** It was utilized to determine how often saline is used during suctioning by nurses and physicians and their knowledge regarding advantages and disadvantages of saline use. It consists of a number of questions on nurses/physicians' use of saline, advantages and disadvantages of its use. A number of proposed statements were listed under each question to select from and a space was left for the participants to add any other statements that are not mentioned.

Tool (II) physiological response parameters assessment sheet: It was developed by the researchers based on extensive literature review **[1,3,7,17].** It was used to collect data related to a number of physiological response parameters. It involves four parts; part (1) involves hemodynamic parameters (HR, SBP, DP, and MAP), oxygenation parameters (pH, $PaCO_2$, PaO_2, HCO_3^-, and SaO_2,), cough response, and lung mechanics (RR, and Cdyn). All these parameters used to compare between the intervention and none-intervention effects. In addition, it involves patient's related characteristics such as; demographic data, current and past medical and surgical history.

Method:

Permission was obtained from the hospital administrative authority to conduct this study after explaining it aim and the process. Tool I was adopted and tool II were developed after reviewing the related literature. Validation of the study tool (II) was assessed by presenting them to five experts from the critical care nursing field. A pilot study was carried out on 5 nurses and 5 patients to evaluate the clarity and applicability of the study tools. They were excluded from the total sample, and the necessary modifications were done.

Data collection, Phase "1":

Tool (I) was distributed to nurses and physicians working in the two ICUs by the researchers after explaining the study aims. Nurses and physicians were asked to complete and return the questionnaire to the nursing office within two days.

Study intervention, Phase "2": Tracheal suctioning was performed only, if there was a clinical need for it. Diameter of the suction catheter was selected not to exceed one half the inner diameter of the artificial airway, providing an internal-to-external diameter ratio of 0.5 in adults **[18]**. In each suctioning episode, three catheter passes only were applied; each of them did not exceed 15 seconds. The catheter was inserted through the tracheal tube until resistance was met, then pulled back slightly. Suctioning pressure was applied only during catheter withdrawal. Immediately before and after suctioning, patients were placed on 100 % oxygen for three minutes and then given 5 breaths with the ventilator. All patients included in the study sample were subjected to the intervention (INS), in which 5 ml of normal saline was instilled before suctioning, immediately before the delivery of the three ventilator breaths given before suctioning. Then, patients will be subjected to the non-intervention (suctioning without saline), four hours after the first intervention, when the patient required again suctioning. Ventilator settings were kept the same during suctioning with and without saline.

Data collection, Phase "2":

Tool (II) was used to collect the following parameters immediately before, immediately after and 5 minutes after suctioning procedure with or without normal saline: HR, SBP, DBP, RR, pH, $PaCO_2$, PaO_2, HCO_3^-, SaO_2, PIP, and Vt. HR was obtained from the cardiac monitor readings. SBP and DBP were measured by a sphygmomanometer manually. An arterial blood gases sample was obtained immediately before, immediately after and 5 minutes after suctioning to determine pH, $PaCO_2$, PaO_2, HCO_3^-, SaO_2. Incidence of cough during suctioning was also documented.

Calculations: Cdyn = Vt/PIP. MAP was calculated as it equals (systolic blood pressure + 2 diastolic blood pressure)/3.

Statistical analysis:

Data were analyzed using SPSS software package version 18.0 (SPSS, Chicago, IL, USA). Quantitative data was expressed using Range, mean, and standard deviation, while Qualitative data was expressed in frequency and percent, McNemar-Bowker was used to analyse the significance between the different stages. Quantitative data was analysed. Not normally distributed quantitative data was analysed using Wilcoxon signed rank test. p value was assumed to be significant at 0.05.

Results:

Phase "1":

Table (1) shows the distribution of the studied sample according to their characteristics. About two thirds of them (68%) are females. Their age ranges from 20 years to 40 years old. Sixteen of them are physicians (14.7%), while 92 of them are nurses (93.3%). Their duration of ICU experience

ranges from 0 years such as those who just started their internship year training to 26 years of experience.

Table (1): Characteristics of studied sample

Characteristic		No. (108)	%
Sex	Male	40	37.0
	Female	68	63.0
Age (year)	Range	20.0 – 40.0	
	Mean ± SD	24.03 ± 4.37	
Position	Practical nurse	14	13.0
	Technical nurse	6	5.6
	Intern nurse	56	51.9
	Nurse supervisor	16	14.8
	Resident physician	12	11.1
	Medical specialist	4	3.6
Years of experiences/year	Range	0.0 – 26.0	
	Mean ± SD	4.19 ± 6.28	

Table (2) demonstrates how often nurses and physicians utilize saline with suctioning. Slightly more than half of nurses and physicians (52%) are utilizing saline frequently with suctioning. Fifty two nurses always or frequently use saline during suctioning; they account 56.5% from the total number of nurses (92 nurses). As for physicians, 12 of them report using saline during suctioning; they account 75% from all physicians (16 physicians).

Table (2):Distribution of studied sample according to their position and utilization of saline

Position	Using saline								Total	
	Always		Sometimes		Rarely		Never			
	No	%	No	%	No	%	No	%	No	%
Practical nurse	0	0	16	23.1	2	6.25	0	0	7	13
Technical nurse	0	0	4	7.7	2	6.25	0	0	3	5.5
Intern nurse	6	75	20	38.5	20	62.5	10	62.5	28	51.85
Nurse supervisor	0	0	6	11.5	6	18.75	4	25	8	14.8
Resident physician	2	25	8	15.8	0	0	2	12.5	6	11.1
Medical specialist	0	0	2	3.8	2	6.25	0	0	2	3.7
Total	8	100	52	100	32	100	16	100	108	100

Table (3) presents nurses and physicians' knowledge regarding advantages and disadvantages of INS. Regarding advantages of the INS, seventy (68.8%) of them (60 nurses and 10 physicians) state that INS stimulates cough reflex. Twenty four (22.2%) nurses and physicians (22 nurses and 2 physicians) report no advantages for the INS. In relation to disadvantages of INS, 68 (64.2%) nurses and physicians indicate chest infection as a complication of INS, while 26 of them (24.5%) report no disadvantages.

Phase "2":

Table (4) represents the characteristics of the studied patients, in which; about two thirds of them are males (65.4%), slightly more than half of them are between 40 and 60 years (53.8%), half of them suffers (50%) from cardiac disorders, and slightly less than half of them (46.2%) have respiratory disorders, and more than half of them (57.7%) have no past history.

Table (5) demonstrates a comparison between hemodynamic response (HR, SBP, DBP, and MAP) to suctioning with and without saline. It is found that HR, SBP, DBP, and MAP do not show any significant change immediately after, or 5 minutes after suctioning in comparison to the before suctioning values , with the absence of any significant difference between suctioning with or without saline.

Table (6) illustrates the comparison between mean differences of change in oxygenation parameters in response to suctioning with and without saline. It is found that pH values significantly increase 5MAS without saline, and decrease IAS with saline (p=0.003, and 0.001; correspondingly). When comparing between pH changes from the base line values in suctioning with and without saline, it is found that there is a significant difference between the two methods of suctioning, in which pH value increases immediately and 5MAS without saline, and decreases immediately and 5MAS with saline (p = 0.04, 0.045, and 0.025; respectively). Although there are no significant differences between $PaCO_2$, PaO_2 and SaO_2 changes after suctioning when comparing between suctioning with and without saline, $PaCO_2$ increases significantly IAS with saline, and PaO_2 and SaO_2 reduce significantly 5MAS with saline (p = 0.04, 0.045, and 0.025; respectively).

Table (7) shows the comparison between mean differences of change in lung mechanics in response to suctioning with and without saline. In relation to respiratory rate values, they increase significantly IAS in both suctioning without and with saline (p=0.021, and 0.032, respectively). Moreover, Cdyn decreases significantly IAS with saline (p=0.013, and 0.005).

Table (8) indicates the incidence of cough or not in response to suctioning with or without saline. This table shows that there is no significant difference between the cough responses to suctioning with or without saline, either immediately or 5MAS.

Table (3) Nurses and physicians' knowledge regarding advantages and disadvantages of INS

Knowledge element		Nurses		Physicians		Total	
		(n=92)	%	(n=16)	%	No=108	%
***Advantage of using saline**	• None	22	23.9	2	12.5	24	22.2
	• Facilitate suctioning large amount of secretions	14	15.22	4	25	18	16.7
	• Stimulate cough reflex	60	65.21	10	62.5	70	64.8
	• Use as doctor order/ for nurses only	2	2.17	0	0	2	1.9
	• Tube obstruction	6	6.52	0	0	6	5.6
***Disadvantages of using saline**	• None	20	21.74	6	37.5	26	24.5
	• Reduce oxygen saturation	12	13.04	4	25	16	15.1
	• Chest infection	60	65.22	8	50	68	64.2
	• Discomfort and pain	30	32.61	4	25	34	32.1
	• Aspiration	6	6.52	0	0	6	5.7

*Multiple responses for more than one choice

Table (4): Distribution of studied cases according to patients' characteristics (n=26)

Characteristic		No.	%
Sex	Male	17	65.4
	Female	9	34.6
Age (year)	< 40	5	19.2
	40 – 60	14	53.8
	> 60	7	26.9
	Range	21.0 – 71.0	
	Mean ± SD	49.12 ± 13.19	
***Diagnosis/disorder**	Respiratory	12	46.2
	Cardiac	13	50.0
	Neurologic	10	38.5
	Renal	3	11.5
	Hepatic	3	11.5
	Metabolic	4	15.4
***Patient's history**	None	15	57.7
	Respiratory	2	7.7
	Cardiac	7	26.9
	Neurologic	2	7.7
	Renal	1	3.8
	Hepatic	0	0.0
	Metabolic	3	11.5

*Multiple responses for more than one diagnosis

Table (5):Comparison between mean differences of change in hemodynamic response to suctioning with and without saline, the immediately and 5 minutes after suctioning values and the before values

Parameter	Without saline			With saline		
	IBS Mean±SD	IAS Mean±SD	5 MAS Mean±SD	IBS Mean±SD	IAS Mean±SD	5 MAS Mean±SD
Heart rate (beat/minute)	95.58 ± 13.79	94.81 ± 19.07	95.15 ± 14.55	97.27 ± 17.22	99.65 ± 22.78	95.77 ± 16.18
p_1		0.455	0.419		0.218	0.861
%change		3.76±22.75	0.69±5.02		1.56±26.80	2.33±15.58
p_2					0.287	0.590
SBP (mmHg)	120.77 ± 28.55	123.62 ± 29.22	124.6 ±26.61	120.19±27.29	127.12±31.25	121.54 ±30.94
p_1		0.589	0.140		0.056	0.772
%change		1.31 ± 12.67	2.94±9.89		4.02±12.37	0.78±14.58
p_2					0.128	0.366
DBP(mmHg)	75.58 ± 14.17	78.08 ± 14.36	76.92 ± 15.10	75.0 ± 18.97	75.38 ± 16.55	72.88 ± 16.38
p_1		0.234	0.321		0.155	0.975
%change		2.50±11.36	0.99±10.06		1.95±26.83	5.36±24.86
p_2					0.394	0.586
MAP(mmHg)	90.064 ± 17.99	93.26 ± 18.54	92.82 ± 17.74	90.06 ± 17.43	92.63 ± 20.33	89.10 ± 20.50
p_1		0.255	0.150		0.077	0.920
%change		2.08±10.97	2.04±8.56		1.25±13.98	3.25±17.15
p_2					0.346	0.231

IBS: immediately before suction, IAS: immediately after suction, 5MAS: 5 minutes after suction
p_1: p value for Wilcoxon signed ranks test between IBS with IAS and 5MAS
p_2: p value for Wilcoxon signed ranks test between the two techniques with the same patients (paired data)
*: Statistically significant at $p \leq 0.05$

Table (6): Comparison between mean differences of change in oxygenation parameters in response to suctioning with and without saline, the immediately and 5 minutes after suctioning values and the before values.

Parameters	Without saline			With saline		
	IBS (Mean ± SD)	IAS (Mean ± SD)	5 MAS (Mean ± SD)	IBS (Mean ± SD)	IAS (Mean ± SD)	5 MAS (Mean ± SD)
PH	7.42 ± 0.11	7.44 ± 0.09	7.45 ± 0.10	7.46 ± 0.11	7.43 ± 0.11	7.45 ± 0.12
p_1		0.866	0.003*		0.001*	0.311
%Change		0.16±0.83	0.32 ± 0.46		0.37±0.50	0.11±0.57
p_2					0.004*	<0.001*
$PaCO_2$ (mmHg)	29.26 ± 8.97	29.65 ± 7.46	28.63 ± 7.08	29.37 ± 9.11	30.82 ± 9.65	30.30 ± 9.32
p_1		0.757	0.576		0.040*	0.148
%Change		2.15±16.22	-1.43±18.41		4.01±11.86	2.33±12.06
p_2					0.328	0.151
PaO_2 (mmHg)	137.89±54.14	139.26 ± 45.57	134.02 ± 54.20	132.52 ± 48.66	123.52 ± 47.98	114.99 ± 51.88
p_1		0.694	0.980		0.137	0.045*
%Change		4.15±46.08	-43.26±192.68		-15.62±38.06	-27.14±48.94
p_2					0.137	0.166
HCO_3^- (mEq/L)	23.99 ± 22.18	19.67 ± 6.72	20.13 ± 6.68	20.79 ± 7.55	20.83 ± 8.24	21.14 ± 8.0
p_1		0.620	0.879		0.666	0.389
%Change		-27.15±149.18	-20.38±124.60		1.50±14.69	0.75±10.37
p_2					0.929	0.990
SaO_2 %	97.37 ± 3.75	98.37 ± 1.45	98.13 ± 2.13	97.77±3.05	96.29 ± 5.22	96.09 ± 5.44
p_1		0.820	0.396		0.071	0.025*
%Change		1.0±3.87	0.73±4.46		-1.80±6.0	-2.05±6.49
p_2					0.107	0.052

IBS: immediately before suction, IAS: immediately after suction, 5MAS: 5 minutes after suction
p_1: p value for Wilcoxon signed ranks test between BS with immediately IAS and 5MAS
p_2: p value for Wilcoxon signed ranks test between the two techniques with the same patients (paired data)

*: Statistically significant at $p \leq 0.05$

Table (7): Comparison between mean differences of change in lung mechanics in response to suctioning with and without saline, the immediately and 5 minutes after suctioning values and the before values

Parameter	Without saline			With saline		
	BS	IAS	5 M AS	BS	IAS	5 MAS
RR (cycle/minute)	22.21 ± 9.10	25.88 ± 9.48	21.19 ± 6.84	26.85 ± 27.43	27.35 ± 11.27	20.96 ± 7.38
p_1		0.021*	0.376		0.031*	0.478
%change		11.66±22.82	-4.70±23.53		11.51±138.34	-41.0±185.91
p_2					1.000	0.523
Compliance (mL/cm H_2O)	29.68 ± 12.25	26.18 ± 10.26	30.0 ± 13.55	29.47 ± 11.42	25.76 ± 10.17	28.06 ± 10.96
p_1		0.124	0.627		0.005*	0.264
%Change		-26.20 ± 68.07	2.01 ± 25.46		-17.49 ± 26.62	-5.97 ± 17.26
p_2					0.909	0.209

IBS: immediately before suction, IAS: immediately after suction, 5MAS: 5 minutes after suction
p1: p value for Wilcoxon signed ranks test between BS with immediately IAS and 5min AS
p2: p value for Wilcoxon signed ranks test between the two techniques with the same patients (paired data)
*: Statistically significant at $p \leq 0.05$

Table (8): Incidence of cough in response to suctioning with or without saline:

Parameter		Without saline	With saline
Cough	Yes (%)	15 (57.7)	17 (65.4)
	No (%)	11 (42.3)	9 (34.6)
@p		0.687	

p: value of McNemar test between suctioning without and with saline
*: Statistically significant at $p \leq 0.05$

4. Discussion:

Phase "1":

Findings of this study phase will be discussed in the following section on the light of other researches regardless the results of phase "2" of the current study. Regarding the INS before suctioning, in the current study, it is obvious that nurses and physicians do not differ a lot in their practices to saline instillation, in which more than half of nurses and three quadrants of physicians always or frequently use saline before suctioning. However, a large number of them reports its risk of chest infection, de-saturation and discomfort, which are reported in many literature [1,19]. This finding may be because nurses and physicians assume that INS has a role in stimulating cough reflex. In accordance with the current study, **Schwenker *et al.,*** study [16], who conducted alike survey on nurses and respiratory therapist, indicated that high responses from respiratory therapists showed that INS stimulates cough reflex. **Schwenker *et al.,*** commented on this finding that if the only benefit of instillation of saline before suctioning is the stimulation of a cough reflex, then INS would not be of a value to a paralyzed patient.

In addition, a number of nurses indicate that they sometimes use saline before suctioning because of the physician's order of utilizing saline that sometimes be written into patient's chart as a routine care. Another factor that leads to frequent use of saline with suctioning is the research findings' conflicts on saline utilization. In line with the current research, **Reeve *et al.*** [20] who conducted a similar survey on the practice of INS, reported that the INS continues to be used by physiotherapists as an aid to clearance of secretions. They attributed this practice as well to the lack of high quality research evidence supporting or not the continuation of this practice. Finally, it could not be also ignored that there are a number of nurses and physicians still believe that INS is risk free and can lubricate secretions, consequently facilitating its removal. However, Ackerman and Mick (1998)[6] indicated that saline and secretions cannot be mixed together. **Halm and Krisko-Hagel** [19] added that the best-known interventions for managing thick tenacious secretions and preventing mucus plugs in ventilator-dependent patients are hydration, adequate humidification, use of mucolytic agents, and effective mobilization instead of subjecting the patient to the harm of saline instillation.

Contrary to the current study, **Schwenker *et al.,*** [16] referred to the inability of most of their research respondent to recognize chest infection as a risk of INS. These differences in perception may be because, in the current study, a large number of nurses are interns who received during their

undergraduate training adequate information regarding saline related risks including; infection and pneumonia. For the physicians, a comment written by one of them is that a recommendation indicated by the last anaesthesia and intensive care conference is not to utilize saline during suctioning as it induces risk of chest infection. So these conference recommendations might raise their awareness to chest infection as a side effect of INS. In conclusion, it is apparent that a considerable number of nurses and physicians can recognize the most common advantages and disadvantages of INS; however, they continue its use before suctioning. Therefore, they need to be more aware about all the complications of INS, and of the alternative techniques to the INS that do not cause any harm to the patient.

Phase "2":

The following section will focus on the comparison between effects of suctioning with and without saline on a number of physiological response parameters. In relation to hemodynamic response, it is found that HR, SBP, DBP, and MAP do not change significantly immediately or 5MAS in both methods and do not differ significantly from method to another. These findings are in accordance with other studies [9, 21] showing that there is no statistical significant difference between the effect of suctioning with or without saline on HR or blood pressure and no significant changes are detected after suctioning. Contrary to these findings, **Akgul and Akyolcu** [22] and **Ackerman** [23] reported a statistical significant increase in HR post suctioning either with or without saline instillation. Also, **Ackerman** [23] reported an elevation in BP after suctioning with saline. Several causes might justify this difference. First, the current study involved several actions preventing suctioning associated hypoxemia which is considered as one of the main causes to hemodynamic changes after suctioning [24]. Second, differences in the way of conducting suctioning procedure are also considered. For example, the technique used for hyperoxygenation, depth of catheter insertion, amount of saline installed may result in different findings. Finally, **Ackerman** [23] emphasized the effect of the statistical analysis test which may result in different findings. They pointed out that in his study, using Wilcoxon signed ranks test did not result in any significant relations in comparison to a two tailed T test.

Regarding changes in oxygenation parameters, the significant decrease in pH values IAS with saline may be related to the significant elevation in $PaCO_2$ associated suctioning with saline, IAS. Furthermore, a statistical significant difference was found when comparing between suctioning with and without saline in relation to the pH value of change. It was found that pH increased IA, and 5MAS without saline, and decreases IAS, and 5MAS with saline. This finding may be due to the elevation of the $PaCO_2$ values IA, and 5MAS suctioning with saline due to inadequate gas exchange secondary to INS that may occlude terminal airways [25]. Contrary to this finding, **Akgül and Akyolcu** [22] found that there is a significant increase in pH following suctioning with saline which was not justified in their study. In relation to $PaCO_2$, PaO_2 and SaO_2 changes after suctioning with and without saline, $PaCO_2$ increases significantly IAS, and PaO_2 and SaO_2 decrease significantly 5MAS with saline which might result from the instillation of saline into the lungs that negatively affects gas exchange [26,27]. In accordance with these results, **Halm and Krisko-Hagel**[19] reported that studies [7, 22, 26, 27] indicated that using normal saline was significantly associated with decreased oxygenation and desaturation that worsened over time after suctioning.

Concerning significant increases in RR occurring IAS in suctioning with and without saline, this finding may be attributed to discomfort and distress caused by the suctioning procedure itself [27]. This is in accordance with **Morrow *et al.*** [28] who reported that spontaneous RR increases significantly after suctioning due to stress and discomfort. In relation to changes in Cdyn, it shows a significant reduction, IAS with saline. This finding is in line with **Cunha-Goncalves** and colleagues [29] who reported a significant decrease in Cdyn after suctioning with saline. This reduction in Cdyn may be attributed to several factors. **Fernandez *et al.*** [25] indicated that the mechanical suctioning procedure itself and the tube disconnection produce atelectasis [28,25]. Dyhr, Bonde, and Larsson (2003) [30], **Martoft *et al.*** [12] and **Fernandez *et al.*** [25] provided other explanation for the observed lung mechanics deterioration following INS; is that saline entrapment causes occlusion of the terminal airways or even micro disturbances in the surfactant system. Therefore they emphasized the need for a lung recruitment maneuver and ventilator adjustments to find optimal PEEP after saline instillation/ suctioning and providing hyperinflation, in order to avoid lung collapse and worsening of lung function and arterial oxygenation. However hyperinflation is controversial due to its possible effect if associated with INS on the incidence of chest infection [8,28-30].

Finally, absence of significant differences between cough response to suctioning with or without saline may prove that INS does not stimulate cough reflex, and the suctioning procedure itself is the stimulator of cough, including; suction catheter insertion and application of negative pressure. This

result is in line with **Gray *et al.*[9]** who reported that cough could be stimulated by the suctioning procedure alone.

Limitations of the study: for phase "1" of the study, the response rate for the physicians to the questionnaire was lower than 50% of them. For phase "2" of the study, static compliance and resistance were not measured because of most of the ventilators cannot measure accurately the flow rate.

5. Conclusion and recommendations:

Based on the findings of this study, it can be concluded that nurses and physicians frequently use saline before suctioning although, a large number of them recognize the most common advantages and disadvantages of INS. Yet, some of them still have inaccurate knowledge regarding INS. Regarding INS, it carry out several risks, including; significant elevation in $PaCO_2$ immediately after suctioning and reduction in oxygen tension and saturation 5 minutes after suctioning. Therefore this study recommends; conducting in-service education to nurses and physicians on this topic raising their awareness on risks on INS and available alternatives to INS. Instead of using normal saline to decrease the viscosity of secretions prior to suctioning, efforts should be made to ensure adequate hydration, adequate humidification, use of mucolytic agents, and effective mobilization. Restoring lung compliance by providing post-suction hyperinflation is necessary to regain lung compliance and prevent atelectasis; however, this recommendation may require further research on its effect on the incidence of infection and hemodynamics. Further researches have to be conducted examining the effect of INS on oxygenation and lung dynamic parameters after 5 minutes of suctioning.

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Spatio-temporal Variations in Phytoplankton Biomass and diversity in a Tropical Eutrophic Lagoon, Nigeria

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Abstract: Taxonomic inventorisation and spatio-temporal variations in the phytoplankton species biomass and diversity, in relation to environmental parameters at the Ologe lagoon, Lagos were investigated from February, 2002 to January, 2004. The annual rainfall, concentrated in one season, initiated increased total solids and nutrient values whereas low dissolved oxygen, conductivity, transparency, and cation concentrations were recorded. The phytoplankton species biomass, composition and water quality indices exhibited seasonal changes closely related to the pattern of rainfall. Estimation of phytoplankton biomass by cell count showed a range of 849 to 1771707cells/ml with mean value of 44052cell/ml. The phytoplankton flora of the lagoon belonged to five main algal phyla, namely Bacillariophyta (84%), Cyanophyta (15.92%), Chlorophyta (0.06%), Euglenophyta (0.018%) and Prryophyta (0.002%). One hundred and nineteen species belonging to forty-nine genera were observed, with diatoms forming the most abundant and diverse. A total of forty-eight species belonging to eighteen genera was found in diatoms. This was followed by green algae, with thirty-two species from fourteen genera, Cyanobacteria, with twenty-three species from eleven genera, euglenoids with seventeen species from five genera, while the dinoflagelates had one species. Nine phytoplankton species were reported to be potentially harmful/toxic bloom species. 57 bio-indicator species were recorded during the period of study. With regard to existing checklist of phytoplankton species, 10 new species are the first reports for Lagos lagoon complex, south-western Nigeria.The centric diatom *Aulacoseira* and cyanobacterium *Microcystis* dominated the phytoplankton community spectrum and their dominance in the Lagoon in both seasons suggests a single floristic grouping. The observed range of bio-indicator species within Ologe lagoon showed that the lagoon is eutrophic. Co-efficient of similarity index indicated that stations close to each other are more similar, than stations further apart.

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Key words: Seasonal changes, phytoplankton composition, cell number, bio-indicator, diversity, eutrophic, rainfall.

1. Introduction

Lagoons are prominent features along the coastal regions of south-western Nigeria. Some of these lagoons are part of West African lagoon system in origin and location but are in form and features similar to freshwater lakes (Alanine 1981). The other types of lagoon are essentially brackish and tidal effects are experience particularly in the dry season. However, all the lagoons of south-western Nigeria enter the sea through the Lagos Harbour. Ologe lagoon is essentially a fishing resource of the people of Lagos and Ogun States. Its location, within these two states and openings into the Atlantic Ocean through Lagos Harbour and Republic of Benin, makes the lagoon national and inter-regional important, in the sense that ecological changes within it, directly affect the productivity and consequently, the fish and fisheries within it and West African coast.

Algological data on the Nigerian lagoons are limited. Of the six lagoons (Mahin, Lekki, Epe, Lagos, Ologe, and Yewa) on the south-western coast, only the Lagos Lagoon has been subjected to extensive published investigations. The available information includes Nwankwo (1986, 1990a, 1990b, 1991a, 1991b, 1994, 1996), Nwankwo and Akinsoji (1989), and Nwankwo *et al.* (1994). Two other papers (Nwankwo and Akinsoji 1992, and nwankwo 1993) extended investigations to other parts of the lagoon system, while currently only one published work on algae in the Epe Lagoon (Nwankwo and Onitiri 1992) dealing with the periphyton algae associated with species of *Ceratophyllum* and *Utricularia,* with special reference to water quality, exist. More recently, Onyema, (2008b), Adesalu, and Nwankwo (2009) investigated the phytoplankton dynamics of Iyagbe lagoon and Lekki lagoon respectively.

There are no previous in-depth biological characterization of the Ologe Lagoon, its physico-chemical regime and the extent and effects of varieties anthropogenic stressors to which the lagoon

and endemic biological resources are exposed to. There is great need therefore for gross understanding of the nature and dynamics of the Ologe lagoon. This is due to the numerous scientific, ecological and economic implications now and for the future. Additionally it is also necessary to study the resident phytoplankton diversity, abundance and distribution in relation to seasonal changes in environmental characteristics operating within the Ologe lagoon.

2. Material and methods

Description of Study Site

Ologe lagoon is a freshwater and non-tidal Lagoon, at the distal end of Badagry creek(low brackish). It is fed throughout the year by the waters of adjoining rivers, creeks, and swamps (fig. 1). The lagoon is located in Lagos State, Nigeria and is one of the nine lagoons in South-western Nigeria (Webb, 1958; Nwankwo, 2004b). It is pressumbaly the smallest of the lagoons in South Western Nigeria with a surface area of 9.4km^2, and lies at the distal end of Badagry creek between longitudes 6° 26'N to 6° 30'N and latitudes 3° 01'E to 3° 07'E. The main body of the lagoon lies within Badagry Local Government Area and it opens up to the Atlantic ocean via the Lagos Harbour and Dahomey in the Republic of Benin. The major source of water are River Owo with a source in a town called Toto Owo where River Ore and Illo form a confluent with River Oponu in Ogun State (Akanni, 1992). Seventeen stations were chosen for sampling within the lagoon.

The lagoon is shallow at most points and is open all year round via the Lagos habour to the sea (Hill and Webb, 1958; Sandison, 1966; Sandison and Hill, 1966). Like all parts of South-western Nigeria, the Ologe lagoon is exposed to two distinct seasons namely the wet (May – October) and the dry (November – April) (Nwankwo, 2004b). Like all parts of South-western Nigeria, the Ologe lagoon is exposed to two distinct seasons namely the wet (May – October) and the dry season (November – April) (Nwankwo, 2004b; Sandison and Hill, 1966). The harmmattan, a short season of dry, dusty North-East Trade winds experienced sometimes between November and January in the region reducing visibility and lowering assemblages is the common macrofloral assemblages especially in areas with reduced anthropogenic influence. The lagoon deposits are varied, and are reflected in the pattern and type of vegetation in the region. Most parts of the Ologe lagoon are colonized by recognizable riparian dense swamp rainforest community dominated by raphia palms especially *Raphia hookeri, Elaeis guineensis, Acrotiscum aureum* and *Cocos nucifera* (Akinsoji *et al.,* 2002).Very few mangrove communities are recognizable around the Badagry creek end. Notable fauna of the area includes amphipods, Oligochaetes, few polychaetes, isopods, barnacles, oysters, periwinkle, nematodes, fiddler crabs, crabs, among others (Sandison and Hill, 1966; Onyema, 2008b).

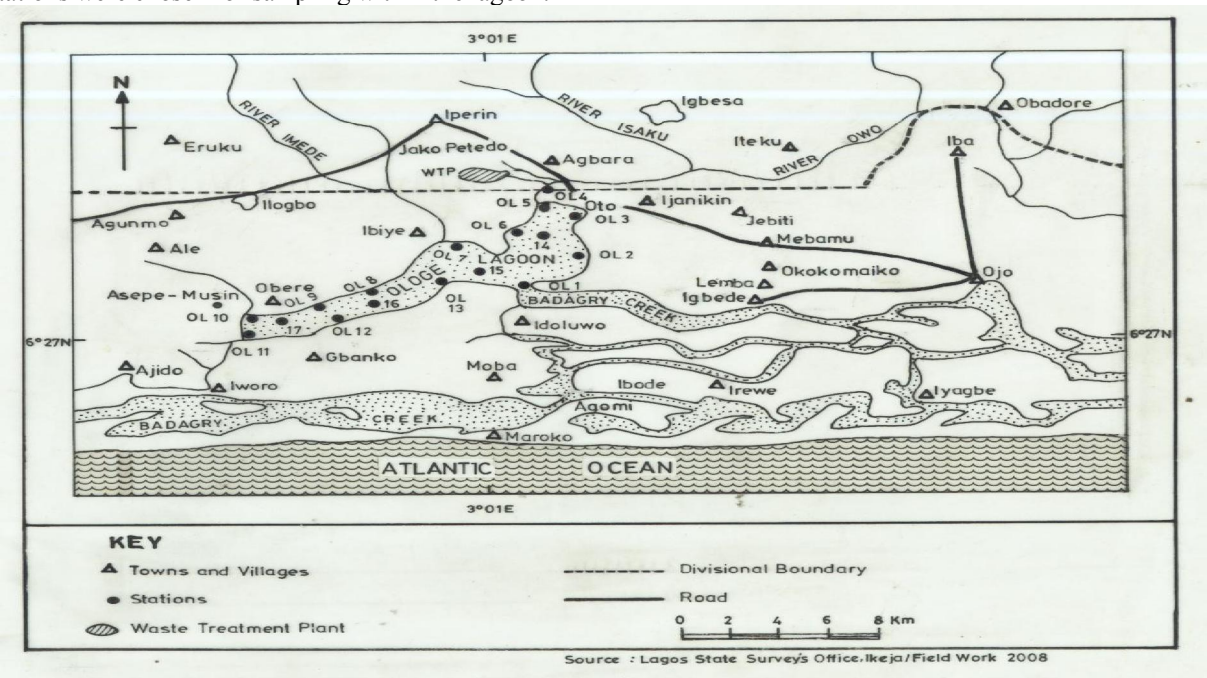

Fig. 1: Parts of Ologe lagoon Showing Sampling Stations

Collection of samples

Collection of water samples

Seventeen sampling stations were selected to cover the lagoon area and for the collection of sample. Table 1 shows the G.P.S location, names and number of sampling stations. Monthly surface water sample was collected for twenty-four consecutive months for physico-chemical characteristics analysis using 500ml plastic containers with screw caps. Collection of samples from the stations was always between 10 and 15hr each time. Water samples were collected just a few centimeters below the water surface at each of the seventeen stations. The plastic containers was then labeled appropriately and transported to the laboratory immediately after collection for further analysis. Water samples for dissolved oxygen was collected also in 50cl bottles and fixed on site with white and black ampoules. Water samples for biochemical oxygen demand (BOD_5) were collected in 200 ml light and dark bottles.Table 2 shows the summary of environmental factors and method/device used for their estimation.

Collection of Phytoplankton Samples

Phytoplankton sample was collected on each occasion and station with a 55 µm mesh size standard plankton net towed from a motorized boat for 5 min at low speed (<4 knots). The net was hauled in and the sample transferred into 250 ml. well labeled plastic container with screw cap. Each sample was preserved with 4% unbuffered formalin and stored in the laboratory. The preserved samples were later taken to Protistology and Aquatic Ecology Research Laboratory, University of Ghent, Belgium for taxonomic studies and scanning electron microscopy. After 48hrs and prior to microscope analysis, samples were concentrated to 10 mL (Nwankwo, 1984).

Biological Analysis

In the laboratory, one drop of the concentrated sample, five different times for each sample was investigated at different magnifications (X100 and X400) using a Wild M11 binocular microscope with a calibrated eye piece. The microtransect drop count method described by Lackey (1938) and employed by Nwankwo (1984) was used to estimate abundance. Since each drop is 0.1 mL and two drops were used for each sample amount, results on abundance were multiplied by 5 to give the values as numbers of organisms per mL. Another sub-sample was acid-cleaned as described by Barber and Haworth (1981) to aid diatom identification. Appropriate texts were used to aid identification (Smith, 1950; Hendey, 1958, 1964; Desikachery, 1959; Wimpenny, 1966; Patrick and Reimer, 1966, 1975; Whitford and Schmacher, 1973; Valandingham, 1982; Nwankwo, 1984, 1990, 2004a; Bettrons and Castrejon, 1999; Siver, 2003; Rosowski, 2003).

Statistical Analysis

Total numbers of species were counted and the species richness estimated according to Margalef (1951, d=(S-1)/InN). The Shannon and Weaver (1949) diversity index ($H=-\sum P_i \ InP_i$) and Pielou (1966) evenness index (J=H/InS) were calculated. The similarities between stations were calculated using the Sorensen index (q=100*2c/(a+b)),while statistica 4.0 was used to calculate ANOVA .

Table 1: G.P.S. locations and station names of sampled areas in the Ologe lagoon

Station No.	Station name	G.P.S. locations
Station OL1	Idolowu	Latitude 6º28'.3 N, Longitude 3º05'.3 E
Station OL2	Between Idolowu and Otto-jetty	Latitude 6º28'.6 N, Longitude 3º05'.5 E
Station OL3	Otto-jetty	Latitude 6º29'.0 N, Longitude 3º06'.0 E
Station OL4	Point of effluent discharge	Latitude 6 º29'.5 N, Longitude 3 º06'.0 E
Station 0L5	Confluence of Owo River and Ologe lagoon	Latitude 6º30'.0 N, Longitude 3 º06'.1 E
Station OL6	Between station 5 and Ibiye	Latitude 6 º29'.3 N, Longitude 3 º06'.0 E
Station OL7	Ibiye	Latitude 6 º29'.0 N, Longitude 3 º05'.6 E
Station OL8	Between Ibiye and Obele	Latitude 6 º29'.2 N, Longitude 3 º06'.9 E
Station OL9	Obele	Latitude 6 º28'.2 N, Longitude 3 º05'.7 E
Station OL10	Asepe Mushin	Latitude 6 º28'.6 N, Longitude 3 º06'.0 E
Station OL11	Gbanko	Latitude 6 º28'.0 N, Longitude 3 º05'.8 E
Station OL14	Centre of Ologe lagoon between otto-jetty and station 6	Latitude 6o ,30'.5 N Longitude 3o 06'.4 E
Station OL15	Centre of Ologe lagoon between Ibiye and Idolowu	Latitude 6o ,30'.2 N Longitude 3o 06'.6 E
Station OL16	Centre of Ologe lagoon between Obele and Ajido	Latitude 6o ,29'.5 N Longitude 3o 06'.0 E
Station OL17	Centre of Ologe lagoon between Asepe Mushin and Gbanko	Latitude 6o ,28'.7 N Longitude 3o 06'.7 E

4. Results

4.1 Physical and chemical features

The minimum and maximum values obtained for the estimates of environmental factors, their means and standard deviation are presents in Table 3. Also in Table 3 is whether each parameter recorded higher values in the wet or dry season for the two (2) years of study. Fig. 2 showed seasonal variations in some environmental factors at some stations in Ologe lagoon from Feb., 2002 to Jan., 2004. Stations represented were selected based on their importance as confluence points and areas exposed to possible anthropogenic stresses or not.

4.2 Phytoplankton

4.2.1 Compositional Abundance

The phytoplankton comprised four groups: Bacillariophyceae, Chlorophyta, Dinophyceae, and Cyanobacteria (Table 1). The Bacillariophyceae had the highest relative abundance and species diversity throughout the year (Fig 3).

Table 2: Summary of environmental factors and method/device used for their estimation.

	Parameter/Unit	Method/Device	Reference
1.	Air temperature (^{o} C)	Horiba U-10	
2.	Water temperature (^{o} C)	Horiba U-10	
3.	Transparency (cm)	Secchi disc method	Onyema 2008
4.	Depth (cm)	Graduated pole	Brown 1998
5.	Rainfall (mm)	Acquired from NIMET, Oshodi, Lagos	
6.	Total Dissolved Solids (mgL^{-1})	Cole Palmer TDS meter	
7.	Total Suspended Solids (mgL^{-1})	Gravimetric method	APHA(1998)
8.	Total hardness (mgL^{-1})	Titrimetric method	APHA(1998)
9.	pH	Electrometric / Cole Parmer Testr3	
10.	Conductivity (µS/cm)	Philip PW9505 Conductivity meter	
11.	Salinity (‰)	HANNA Instrument	APHA(1998)
12.	Alkalinity (mgL^{-1})	Titration method	APHA(1998)
13.	Dissolved oxygen (mgL^{-1})	Titration method	APHA(1998)
14.	Biological oxygen demand (mgL^{-1})	Incubation and Titration	APHA(1998)
15.	Chemical oxygen demand (mgL^{-1})	Titration method	APHA(1998)
16.	Nitrate – nitrogen (mgL^{-1})	Colorimetric method	APHA(1998)
17.	Phosphate – phosphorus (mgL^{-1})	Colorimetric method	APHA(1998)
18.	Silica (mgL^{-1})	Colorimetric (DR2010)	APHA(1998)
19.	Sodium (mgl-1)	Flame Photometer	APHA(1998)
20.	Potassium (mgl-1)	Flame Photometer	APHA(1998)
21.	Calcium (mgL^{-1})	Titrimetric method	APHA(1998)
22.	Magnesium (mgL^{-1})	Titrimetric method	APHA(1998)
23.	Copper (mgL^{-1})	Atomic Absorption Spectrophotometer Perkin Elmer 5000 AAS	Perkin Elmer Application (2002)
24.	Iron (mgL^{-1})	Atomic absorption Spectrophotometer Perkin Elmer 5000 AAS	Perkin Elmer Application (2002)
25.	Zinc (mgL^{-1})	Atomic Absorption Spectrophotometer Perkin Elmer 5000 AAS	Perkin Elmer Application (2002)
26	Chromium (mgl-1)	Atomic Absorption Spectrophotometer Perkin Elmer 5000 AAS	Perkin Elmer Application (2002).

Table 3: A summary of the minimum, maximum and mean / standard deviation estimate values for environmental factors from the Ologe lagoon (February, 2002 – January, 2004).

	Parameter/ Unit	Minimum value	Maximum value	Mean value ± S.D.	**Higher values reported in t** In the---
1	Air temperature (oC)	27	34	31.10 ± 0.22	Dry season
2	Water temperature (oC)	25.2	31.8	29.01 ± 0.47	Dry season
3	Transparency (cm)	24	76	51.54 ± 5.65	Dry season
4	Depth (m)	3.2	7	4.4	Wet season
5	Total Dissolved Solids (mgl-1)	48	294	139.23 ± 17.89	Dry season
6	Total Suspended Solids (mgl-1)	7	378	184.36 ± 14.90	Wet season
7	Rainfall (mm)	0.6	383	137.37	Wet season
8	Total hardness (mgl-1)	62	342	146.38 ± 26.52	Dry season
9	pH	5.8	8.1	6.92 ± 0.14	Dry season
10	Conductivity (µS/cm)	83	621	256.59 ± 36.65	Dry season
11	Salinity (‰)	0.0	0.5	0.10 ± 0.03	Dry season
12	Alkalinity (mgl-1)	42	162	100.20 ± 9.37	Dry season
13	Dissolved oxygen (mgl-1)	7	12.7	9.08 ± 0.42	Wet season
14	Biological oxygen demand (mgl-1)	0	28	13.11 ± 1.79	Dry season
15	Chemical oxygen demand (mgl-1)	6	39	21.34 ± 2.52	Dry season
16	Nitrate – nitrogen (mgl-1)	0.02	1.02	0.44 ± 0.08	Wet season
17	Phosphate – phosphorus (mgl-1)	0.03	1.79	0.80 ± 0.10	Wet season
18	Silica (mgl-1)	2.05	9.54	5.07 ± 0.45	Wet season
19	Sodium (mgl-1)	2.6	22.7	30.82 ± 6.13	Dry season
20	Potassium (mgl-1)	0.1	7.6	8.71 ± 1.78	Dry season
21	Calcium (mgl-1)	34	227	91.27 ± 17.89	Dry season
22	Magnesium (mgl-1)	0.01	7.6	2.64 ± 0.62	Dry season
23	Copper (mgl-1)	0.02	0.06	0.03 ± 0.001	Dry season
24	Iron (mgl-1)	0.12	0.99	0.35 ± 0.04	Dry season
25	Zinc (mgl-1)	0.002	0.03	0.01 ± 0.001	Dry season
26	Chromium (mgl-1)	0.001	0.04	0.02 ± 0.002	Dry season

The mean values of the physico-chemical factors of the investigated stations clearly indicated a seasonal trend resulting from the concentration of rainfall in the period from May to November (fig. 2).

Table 4. Mean density of phytoplankton (ind. mL $^{-1}$) in Ologe Lagoon from February 2002 to January 2004.

Taxa	Seasons		
	Jan-Apr.	May-Sep.	Oct.-Dec.
BACILLARIOPHYCEAE Centric			
Aulacoseira granulata (Ehr.) Sim.	10400-50200	2250-25200	32500-72500
A. granulata var.*angustissima* (O.F.Mullar) Sim.	3700-6180	5100-8850	9850-11220
**A. granulata* var.*angustissima f.spiralis* Hust.	1420-4650	880-2260	2660-6100
**A. granulata* var.*angustissima f.curvata* (Hust.)	1600-5040.	1020-2040	2250-5670
A. granulata var.*muzzaensis* (Meist.) Hust	2490-4880	640-2840	3600-6680
**A. islandica* (O. Muller)	1020-2140	150-400	1400-3660
Stephanocyclus sp	10-30		
Cyclotella meneghiniana (Kutzing)	40-50	6-10	50-120

C.striata (Kutz.) Grunow			6-20
C.stelligera Cleve ex Grunow		8	
Coscinodiscus centralis Ehrenberg			4-20
C. eccentricus Ehrenberg	6		10-20
**Melosira varians* Agargh		10	
Actinoptychus sp	4-15		
**Biddulphia laevis* Ehrenberg		2-10	2-6
ORDER 11: PENNALES			
Synedra ulna (Nitzschia) Her	10-30	40-120	20-60
S.acus Kutzing	8-20	30-70	40-60
Nitzschia palea (Kutz) W.M.Smith	8	20-30	10-20
N. closterium (Ehr.) W.M.Smith		10	6
N. acicularis (Kutz.) W.M.Smith		4-15	
N.vermicularis Hantzsch		4-10	2
Pinnularia major (Kutz.) Cleve	8	20-40	10
P. interrupta W.M.Smith		2-12	
P.laevis (Ehr.) Compere		10-30	
P.hemiptera (Kutz.) Rabenh.	2-6	4-16	
P.ambigua Cleve	6-8	20-30	6-10
Pinnularia sp		15-25	
Navicula oblonga Ehrenberg		20-40	10-20
N. radiosa Kutzing		6-14	8
N. gracilis Ehrenberg	4	6-10	
N. mutica Kutzing		8	4
N. cuspidata Meist		4-10	2-4
Cocconeis placentula (Ehr.) Cleve		8	
C. Disculum (Schum) Cleve			4
*Epithemia sp	2-8		2-10
Cymbella affinis Kutzing	6-20	10-30	10-20
C. minuta Hisle ex.Rabenh	8		
Eunotia gracilis Meister		8-20	2-10
E.lunaris (Ehr.) Grunow		6	2
E.monodon Ehrenberg	4		
Surirella elegans Ehrenberg	10-20	10-50	8
S. ovata Kutzing	6		
Fragilaria construens Ehrenberg	4-10	10-40	
**Gomphonema parvulum* Kutzing	8		4-30
DIVISION: CHLOROPHYTA			
CLASS: CHLOROPHYCEAE			
ORDER I: CHLOROCOCCALES			
Pediastrum simplex (Meyer) Lemm	4-40	15-40	10
P. simplex var.echinulatum (Wittr.)		6-14	
P. duplex Meyer	4-10	10-50	8-20
P. duplex var.gracillimum (W. West)		6	

P. tetras (Ehr.) Ralfs		4-15	
P. tetras var. tetraodon Rabenhorst		4	4
*P. boyanum (*Turpin) Meneghini		4	
Scenedesmus acuminatus (Lagerh.) Chordat	8-50	6-30	2-10
S. quadriacuada (Turp.) Breb	4	2-10	6
**S. dimorphus* (Turp.) Kutzing		4	
S. apiculatus (W.et G.S.West) Chordat		8	2-10
S. arcuatus Lemm.	4	2-10	
Ankistrodesmus acicularis (A.Braun) Korsh.	2	10-30	4-15
A. falcatus (Corda) Ralfs.			6
Tetraedron sp	2	10-20	
ORDER II:VOVOLVOCALES			
Volvox aureus Ehrenberg	20-50		10-20
V. africana Ehrenberg	8-20		
Eudorina elegans Ehrenberg	4-20		20-30
ORDER III: ZEGNEMATALES			
Staurastrum leptocladium Nordst	10-20	2-10	10-40
Staurastrum paradoxum Meyen	6		
Staurastrum sp	2-10		8
Desmidium swartzii Ag		2-8	2
Micrasterias sp		2-20	
Spirogyra africana (Fritsch) Czurda	4		
Zygnema sp		8	6
Closterium ehrenbergii Menegh		10-40	6-20
C. aciculare T.West		4-10	4
C. kuetzingii Breb.	4-10	6-40	6-20
C. intermidium Ralfs			4
C.moniliferum (Bory) Ehr.Ex Ralfs		6-20	2-10
Spondylosum sp		2-4	
Cosmarium sp		4	
DIVISION: EUGLENOPHYTA			
CLASS: EUGLENOPHYCEAE			
ORDER: EUGLENALES			
Euglena acus Ehrenberg	10-20	4	4-10
E. caudata Hubner	4-10		2-6
E. convoluta Korishikor	6-14	2	2-8
E. viridis	4-15		4-6
E. polymorpha Dangeard	2-8	4	2-10
Euglena spirogyra Ehrenberg	6	2	2-6
Lepocinolis sp	6		
Phacus accuminatus Stokes	8-20	2-4	4-15
P. longicauda Duj.	4-8		2

P. orbicularis Hubner	6		
P. curvicauda Swir	4		
P. tortus (Lemm) Skvort	2-8		4
Trachelomonas caudata stein	12-25		4-12
T. hispida Lemm	2-4		
T. armata (Ehr.) Stein	6		
T. acanthostoma (St) Deft			4
Eutreptia sp	2-6		
DIVISION: CYANOPHYTA			
CLASS: CYANOPHYCEAE			
ORDER I: CHROOCOCCALES			
Microcystis aeruginosa Kutzing	3300-8450	820-2800	4950-12420
M. flos-aquae Kirchn.	1660-4400	1060-2050	2660-6110
M.wesenbergii Komark	650-970	240-400	820-1020
Merismopedia glauca Ehr. Nag.	350-510		
Gloeocapsa decortisans	40-80		20-60
ORDER II: HORMOGONALES			
Spirulina major Kutzing	420-1500	100-250	400-2110
S. princeps W.et G.S.West	30-50		50-150
S. platenensis Geitler	160-450	40	200-550
Spirulina jenneri Geitler	320-850	60-120	450-1200
Aphanocapsa sp	30-50		20-60
Aphanothece sp	10-30	8	5-20
Anabaenopsis sp	12		10
Anabaea spiroides Klebahn	280-400	30-60	
A. flos-aquae Elenkin	1200-2000	40-120	1650-2500
Lyngbya contorta Lemm.	30-70	120-200	50
L. circumcreta	10-30		
L. limnetica Lemm	10-20		30-120
**Oscillatoria formosa* Bory	20-30	30-120	10-20
O. limnetica Lemm.	8-20		
O. nigro-viridis Thwaites		8-20	
Nostoc sphaerica Vaucher	30-60	40-120	20-50
**N. linckia* Bornet et Thuret		10-30	
**N. caeruleum* Lyngbye	8	4-20	10
DIVISION:DINOPHYTA			
CLASS:DINOPHYCEAE			
ORDER:PERIDANALES			
Peridinium cintum (O.F. Mull.) Her			2

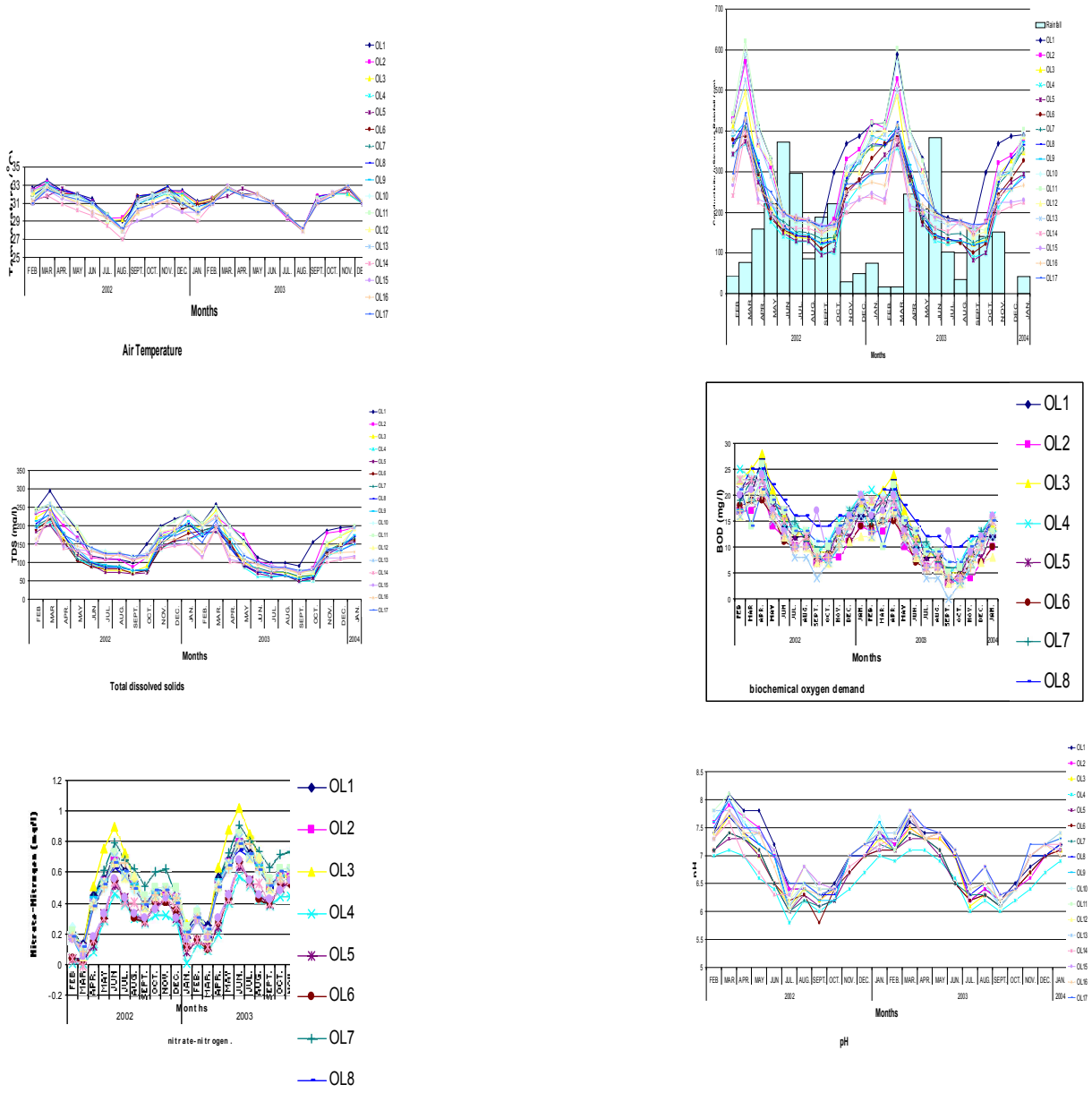

Fig 2: Seasonal changes in the physico-chemical parameters in the Ologe Lagoon, Lagos

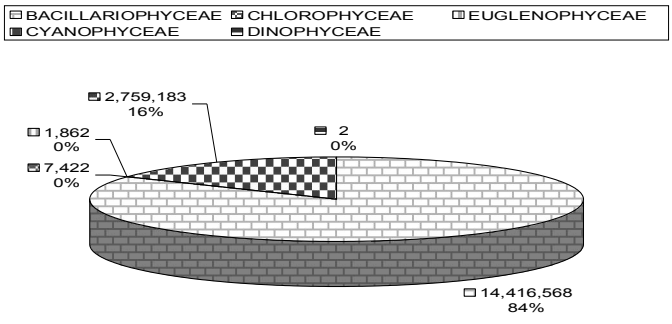

Fig 3: Total Percentage Compositions of the different Classes of Phytoplankton species at Ologe lagoon, Lagos.

Cemtric diatoms were quantitatively dominant in the Ologe Lagoon throughout the year but in terms of species richness, the Pennate were more abundant and prevalent during the raining season. The commonest species were *Aulacoseira granulata, A. granulata* var. *angustissima, A. granulata* var. *angustissima f. spiralis, A. granulata* var. *angustissima f. curvata* and *A. islandica* and they made up to 70% of the total diatom cell counts.

Pediastrum simplex, P. duplex, P. boryanum, Eudorina elegans , Scenedesmus acuminatus, Scenedesmus quadricauda and their varieties were the most prevalent rheophilic green algae. Filamentous desmids such as *Desmidium swartzii, Staurastrum leptocladiu, Closterium ehrenbergii, C. kuetzingii* and *C.moniliferum* were prominent species in the dry season.

The euglenoids were more abundant and prevalent during the dry season. Most common occurring species species were *Euglena acus, E. caudata, E. convoluta, E. polymorpha, Phacus accuminatus,* and *Trachelomonas caudata*

The most dominant Cyanobacteria were *Microcystis aeruginosa, M. flos-aquae,* and *M. wesenbergii.* Their share in terms of number was high in the dry season, when these chroococal taxa reached their maximum, and immediately after the rainy season of each year. Filamentous forms (genera Synedra, *Anabaena, Lyngbya, Aphanotheca, Aphanocapsa, Anabaenopsis, Oscillatoria, Nostoc* and *Spirulina* were frequent but in smaller numbers.

The member of Dinophyceae, *Peridinium cinctum* was rarely and therefore its contribution to the total phytoplankton density was insignificant.

4.2.2. Seasonal variations

In the dry season (January-April) the diatom cell counts were high during the study period. Centric diatoms (especially *Aulacoseira* spp.), chroococcal Cyanobacteria (*Microcystis* spp.), constituted more than 91.1% of the total density. Between January and April each year *Microcystis* spp. increased from 650 to 8450 cells mL^{-1}.Filamentous desmids ranged from 10 to 30 cells mL^{-1}, while *Aulacoseira* spp. ranged from 1020 to 50200 cells mL^{-1} in April. During this period many of the Pennatae diatoms though frequent, maintained small numbers, but green algae (*Eudorina, Volvox, Pediastrum, Scenedesmus*) attained their maxima.The euglenoids were more prevalent and abundant during this period.

The period May-september is characterized by the prevalence of non-motile green algae *(Ankistrodesmus, Tetraedron, Closterium*). Generally, this was a period of low phytoplankton biomass. For instance, *Aulacoseira* species dropped from 2840 cells mL^{-1} in May to 150 cells mL^{-1} in July but increased again to 32500 cells mL^{-1} in October. Biomass of Cyanobacteria dropped during this period, *Microcystis* sp. From 2800 cells mL^{-1} in May to 240 cells mL^{-1} in September, while Hormogonale Cyanobacteria and the euglenoids followed similar trends with some of them being absent this period.

After the low counts observed in the wet months, between October and December significant increases were recorded. *Microcystis* spp. increased from 820 cells mL^{-1}in October to 12420 cells mL^{-1} in December. Among the diatoms *Aulacoseira* spp. increased from 1400cells mL^{-1} to 72500 cells mL^{-1} while in green algae had Pediastrum spp., Scenedesmus spp. and *Staurastrum leptocladium, Closterium spp.* and most euglenoids as some of the prominent taxa.

Fig 4 below showed the spatio-temporal variations in the total count of phytoplankton species among the stations during the period of study, while fig 5 showed the spatial distribution of different classes of phytoplankton species at Ologe lagoon. The values for this parameter ranged between 849 cells/ml and 177107 cells/ml among all the sampling stations with a mean value of 44052 cells/ml for the duration of study. The highest total count (177107 cells/ml) was recorded at station OL10 (Asepe Mushin) in the month of December 2002, while the lowest value (849 cells/ml) was observed at station OL4 (point of effluent discharge) in the month of May 2003. Mean values for this parameter were comparatively higher during the dry season than the wet season among all sampling stations during the study period.

4.2.3 Species diversity

Diversity was comparatively lower in the dry season than during the rainy one with the exception of Margalef index which recorded highest index in February (fig. 5).The number of taxa (species and varieties) and abundance were higher in the dry season than the rainy season. The compositions of phytoplankton were relatively similar at all the stations of the Ologe lagoon. Between Station 1 and 11 similarity values of more than 90% were recorded, suggesting the control of similar forcing functions.

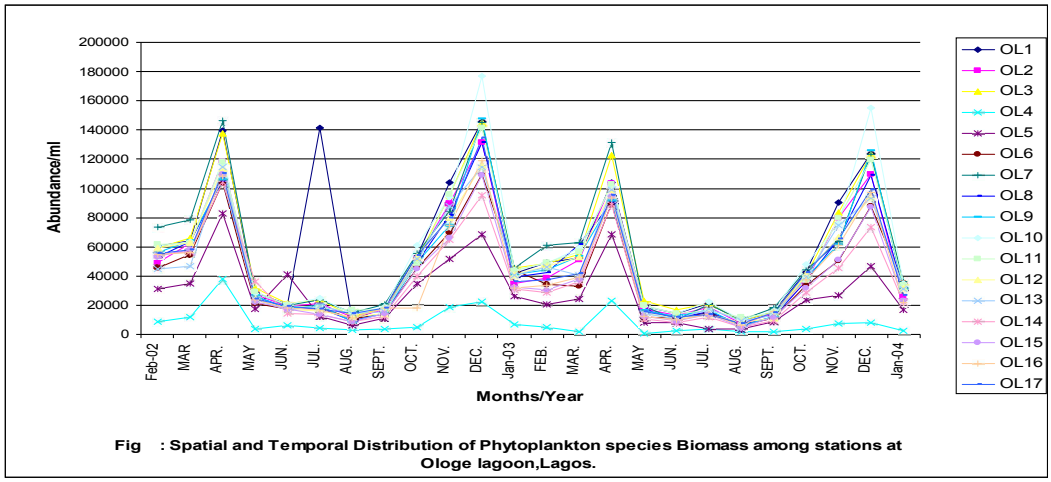

Figure 4. Spatial temporal distribution

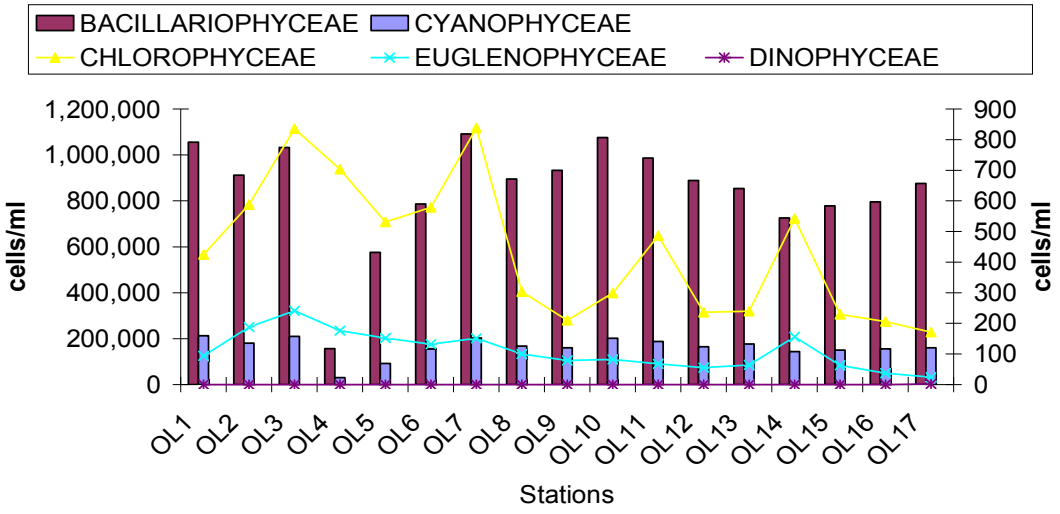

Fig 5: Spatial distribution of different classes of phytoplankton species at Ologe lagoon, Lagos

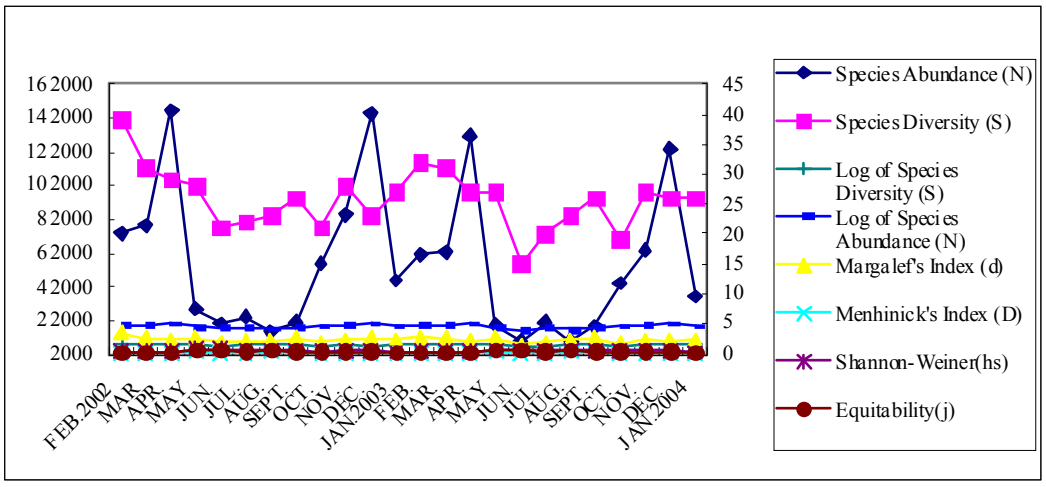

Fig 6: Some community composition parameters at Ibiye station in Ologe Lagoon, Lagos.

5. DISCUSSION

The ecological factors operating in the lagoon of South-western Nigeria have been documented by several workers (Hill and Webb, 1958; Olaniyan, 1969; Ezenwa, 1878; Nwankwo, 1984; Solarin, 1985; Onuoha et el, 2010). The observed physico-chemical parameters showed that, although Ologe lagoon is part of Lagos lagoon complex, in origin and location, it remained freshwater throughout the year ,due to it's distance from the Lagos harbour and constant influx of water from the rivers, adjoining creeks and swamps. Sandison and Hill (1966), noted that there was obvious decrease in maritime influence as points from the lagoon were increasingly distant from the harbour. According to Sandison and Hill (1966), all the waters entering from the west of the Lagos harbour flow through the Badagry creek and the harbour forms the main outlet to the sea for the brackish and fresh water flowing through the marginal lagoon system of South-western Nigeria. Nwankwo and Onitiri (1992), observed that rainfall and flood waters were the most important factors operating in a distant lagoon from the Lagos harbour. According to them, these factors act as forcing function by introducing nutrient through flood waters which affects the chemical environment. The flood effect determined the rate at which resident phytoplankton species within the lagoon were pushed westwards towards the sea. A similar flood effect on periphyton assemblages in the Epe lagoon has been reported by (Nwankwo and Onitiri, 1992).

The physico-chemical parameters observed in the lagoon exhibited seasonal changes that are closely related to the distributive pattern of rainfall of the region. According to Brown and Kusemiju (2002), rainfall pattern in the tropics creates the dry and wet season experienced in West Africa.For instance the raining season concentrated between May and October was accompanied by low conductivity, pH, temperature, transparency, cations, total dissolved solids, salinity, total alkalinity, biochemical oxygen demand, chemical oxygen demand and total hardness.On the other hand,the total suspended solids,total solid, dissolved oxygen, and nutrients levels increased (Sandison,1966; Sandison and Hill,1966; Nwankwo1996).Past researchers had observed that hydrological conditions and the phytoplankton spectrum of the Lagos and Epe lagoons are governed by the rainfall events and tidal seawater inflow (Hill and Webb, 1958). Seasonal and spatial changes in the phytoplankton biomass and community were recorded during the two year seasonal cycle and were in response to changes in environmental factors. There were clear differences in phytoplankton species biomass between stations within Ologe lagoon further off the effluent discharge station and stations closer to, or on it during the different seasons. The phytoplankton species biomass ranged between 849 cells/ml and 177107 cells/ml among all the sampling stations with a mean value of 44052 cells/ml for the duration of study. The highest total count (177107 cells/ml) was recorded at station OL10 (Asepe Mushin) in the month of December 2002, while the lowest value (849 cells/ml) was observed at station OL4 (point of effluent discharge) in the month of April. Investigating phytoplankton dynamics as influenced by effluent discharges from Agbara industrial and residential estates and other human activities will give an in-sight into the exploitation of various microhabitats offered by the underlying causal factors.

The seasonal variations in the composition of the observed phytoplankton species were similar to that of the rest of the lagoon system (Nwankwo,1996) and probably falls into a single floristic grouping dominated by the species of genera of centric diatom *Aulacoseira* sp and cyanobacteria (*Microcystis* sp). Similar dominance of diatoms among phytoplankton assemblages have been reported by other ecologists in the coastal waters of Nigeria (Nwankwo and Onyema, 2004; Onyema and Nwankwo, 2006).

Phytoplankton densities were higher in the dry season than the wet (rainy) season. Ezra and Nwankwo (2001) also recorded higher cell densities in phytoplankton in the dry season than was in the wet season in Gubi reservoir, Bauchi state.Similar observation was made by Nwankwo(1998) in Epe Lagoon. Some of the recorded taxa such as *Microcystis aeruginosa, M.flos-aquae, Anabaena spiroides, Aulacoseira granulata, A. granulata* var. *angustissima, Desmidium swartzii* and *Ankistrodesmus* sp and euglenoid species are indicators of organic pollution and have been associated with eutrophic waters elsewhere. They may have existed in the Lagoon owing to increased influx of nutrients through Agbara industrial effluents and other human activities.

Analysis using ANOVA showed significant differences in the sample means of physico-chemical parameters of effluent discharge station (OL4) and the other stations within the lagoon at 5% level of probability. Changes in species diversity and phytoplankton abundance are attributed to changes in rainfall pattern and its associated influx of floodwaters. Comparatively, lower species diversity recorded during the dry season than the wet season was in consonance with Nwankwo (1996) for the Lagos lagoon. Co-efficient of similarity index

indicated that stations close to each other are more similar, than stations further apart. There was also, dissimilarity among stations in the different seasons of the year. Nine phytoplankton species were reported to be potentially harmful/toxic bloom species (Hallegraeff *et al*., 1995; Nwankwo et al., 2003).

The seasonal data which were based on cell counts may be an accurate reflection of biomass. The main growth period appeared to begin immediately after the rains, this being associated with increased nutrients, decreasing flood conditions, and increasing transparency. The prevalence of pennate diatoms in the rainy season may be due to their recruitment from the littoral, rivers, and adjoining swamps. The present observation that diversity varied with the season and changes in phytoplankton composition agrees with observations made in the Lagos Lagoon (Nwankwo 1996). Following the use of the above community structure indices, a biological appraisal of that lagoon was satisfactorily done. Between November and December, the period immediately after the rains, there was the *Aulacoseira-Microcystis* assemblage while between January and April, the *Aulacoseira-Microcystis*-euglenoids assemblage dominated. The dominance of these taxa may have accounted for the low diversity indices recorded during the study period.

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Self-esteem and quality of life among visually impaired children in Assiut City, Egypt

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Abstract: Nowadays, quality of life is one of the important aspect in programming and giving service to disabled and blindness is one of the most important and common kinds of physical handicaps. This study aimed to: determine Self-esteem & quality of life among visual impaired children in Assiut City. Descriptive cross sectional research design was used in this study. The studied children were 100 children from El-Noor School, Assiut City, 22 children from last two years of primary school, 44 children from preparatory school, 34 children from secondary school with age ranged from 12 to 18 years and both sex. This study is divided to four tools; the first tool was structured interview sheet included biosocial data of children, time and causes of disability, second tool to assess social class by using Abd-El-Twab scale 1998. The third tool - Self-esteem scale, it includes 10 items. , the final tool to assess quality of life data, it consists of 68 items. Following approval for the research, personal meetings were held with each child in school sitting. Data was collected during the period from first of September 2009 to the end of April 2010. Obtained results revealed that more than two-thirds of studied children aged between 12-18 years. The mean age of studied children is 15.87 ± 3.03 and more than half of them were males. Nearly three quarters of studied sample had low social class (72%). In addition; nearly half of children with high total quality of life score were come from high social class compared with 8.3% from low social class children with highly statistically significant difference. Also, it was found that 40% of studied children with high total quality of life scores were outdoor school residence compared to only 8.2% of indoor school residence (p -value 0.003). Further, more than half of studied children with low social class had low self-esteem compared to more than three - quarters of normal self - esteem children were come from high social class. 71.4% of normal self-esteem children were present in small family compared by two third of low self-esteem score were present in large family**.** It is concluded that children from high social class had higher quality of life and normal self esteem than other children**.** It is recommended that multicomponant intervention with a focus on the children and their families are needed to improve self esteem and quality of life of visually impaired children. Designing and implementing of a psychological counseling program for visually impaired children to improve their emotional statues and help them to coping effectively.
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Key words: Visually impaired, Self-esteem, quality of life, school age children.

1. Introduction:

One of the most common and important group of handicaps, are blinds international health organization, on the international day of blindness in year 2002 announced that about 45 million blind live in the world every 5 minutes, one person becomes blind in the world. Rapidly increasing their population, the importance of blinds welfare becomes obvious **(Dehestani, 2004).**

Childhood blindness remains a significant problem, with an estimated 1.4 million blind children below age 15 and more than 90% of the worlds visually impaired live in developing countries. The first global estimate on the magnitude and causes of visual impairment was based on the 1990 world population data, 38 million blind. This estimate was later to the 1996 world population (45 million blind), and to the projected 2020 world population (76 million), indicating a twofold increase in the magnitude of visual impairment in the world by 2020. In Egypt blind children were 0.25% of children and low vision children constituted 0.1% of total children in 2001 **(WHO, 2004).**

Visually impaired school age children have the same rights as non-disabled people living in the wider community. These rights include socializing sexuality, learning, economic independence, dignity and respect access to community services, marriage and raising a family. Disabled people need to be productive members in their communities **(WHO, 2004).**

Quality of life is a broader concept than personal health status, it involves four dimensions. The first one is health and physical functioning it includes activity level, mobility, physical symptoms, ability to take care of responsibilities and participation in recreational activities (Mayou and Bryant, 1993), the second dimension is psychological and spiritual attitudes and responses which include satisfaction of life, anxiety, stress self-esteem achievement of goals, purpose in life, spiritual aspects; religion, sense of security and control over

own life. The third dimension is social and economical involvement, which focus on employment work, education, financial status, friendship and social support. The last dimension is family relationships which include relationship with spouse, relationship with children and family happing **(Walker and Rosser, 1997).**

In terms of global definition, quality of life consists of satisfaction with life. Personal feelings of well being or happiness, satisfaction is usually associated with cognitive dimensions and happiness with emotional ones **(World Health Organization, 1999, Abd El-Aziz and Ibrahim, 1995).** On the other hand, medicine and nursing science define quality of life as the one involving health and functional ability. Health related quality of life includes health care, health habits, health knowledge and attitudes, use of health services, persons social networks and their coping skills plus the economical, educational and psychological resources **(Lukkarinan et al., 1997).**

Quality of life is defined as an individual perception of his or her position in life in the context of the culture and value system in which he or she lives and in relation to his or her goals, expectations, standards and concerns. This definition highlights the views that quality of life refers to a subjective evaluation which induces both positive and negative dimensions and which is embedded in a cultural, social and environmental context **(Haber and Krainovich-Miller, 1997).**

Self-esteems generally considered the evaluative component of the self-concept a broader representation of the self that includes cognitive and behavioral aspects as well as evaluative or affective ones. It is a widely used concept it refers to an individual sense of his or her value or worth or the extent to which a person values approves of appreciates prizes or likes him or herself **(Blascovich and Tomaka, 1991**). The most broad and frequently cited definition of self-esteem within psychology is Rosenberg's (1965), who described it as a favorable or unfavorable attitude toward the self.

The rehabilitation nurse seeks to promote a quality of life that meets the client's definition of dignity and promotes self-respect and self-reliance. The quality of life is influenced by several interrelated factors such as personal and family purpose and goals, functional abilities, social supports, communication skills, activities of daily living, cognition, problem solving and decision-making skills, pain and comfort economic resources, and basic human requisites food shelter, and safety. The rehabilitation nurse considers these factors during the assessment of needs, establishment of goals, and development of intervention strategies. Rehabilitation is a family centered process. The disabled individual who is part of family is viewed as a subsystem of a living dynamic system with group core process, needs, expectations, and roles. Rehabilitation nurses recognize that families must be equipped with knowledge and skills to support a disabled member, also, achieves wellness by taking actions to reduce functional limitations. Interventions are designed restore, maintain, and promote healthy lifestyles for disabled individuals **(Shirle and Jill, 2001).**

Aim of the study:

The study aimed to determine Self-esteem and quality of life among visually impaired children.

2. Subjects and methods:

Research design: descriptive cross sectional research design was used to conduct this study

Setting: the study was conducted at the El-Noor School at Assiut City, Egypt

Subject: The total number was 100 children from El-Noor School at Assiut City. The children aged from 12-18 years and both sex. All children from preparatory and secondary school were included (44 and 34 respectively) and all children last two years of primary school (22) who understand the questionnaire (Age of entrance the school above 7 years).

2. Methods of data collection:

Following approval for the research program by the Ministry of Education research committee, personal meetings were held with each of the subjects. Each student answered a questionnaire individually. Fours tools were used in this study.

Tool 1:

A structured questionnaire was used to collect socio-demographic data of children, , it include age, sex, residence, number of friends, grads, number of children in the family, birth order, time and causes of disability.

Tool 2:

Socio-economic scale; it was developed by (Abd El-Twaab 1998); used to assess the socio-economic status of the visually impaired children. It included four items; level of education of parents (8 items), family income (6 items), job of parent, life styles (3 items). Each item have one score the total score were divided into three classes as high, moderate and low class. It has been modified the item of income of social class by the researchers as following; according to the rate of inflation and increase to be conforming with recent income

through comparing difference of the value of the golden pound at 1998 to that at 2008 and multiplying the rate of inflation to the scale

Tool 3:

Self-esteem scale, the original scale was constructed by Rosenberg Self-esteem (1965) it include 10 items that are usually scored using a four point responses ranging from strongly disagree=0, disagree=1, agree=2, and strongly agree=3, the total score was thirty point, children who obtained score less than 15 degree were considered to have a low Self-esteem while those who scored between15-30 degree were considered to have a normal self-esteem.

Tool 4:

Quality of life scale, it consists of (68) items divided into six domains or subscales. First subscales, comprising 13 items covering the physical health. The second subscales, consisted of 17 items reflecting self-care (self depended). The third subscales, included 6 items representing child emotional status and religions, the fourth one consisted of 13 items related to personal and social relationships, the fifth subscale, consisted 6 items which assess the environment and ability to take decision. The last subscale, consisted of13 items used to collect data about spiritual concerns and personal beliefs. Quality of life; the original scale was constructed by Lehman (1986) and world health organization, (1997) to assess QOL. The scale was modified by the researchers to measure the current concept under study. Responses were measured on a three points likart scale ranging from 0-2. in which the higher score, the better QOL for every answer, always = 3, sometimes =2 and rarely =1. The scoring was reversed for negative items The total score was 204 point, children who obtained score less than 78 point were considered to have a low QOL while those who scored between102-153 were considered to have a moderate QOL and finally those who scored more than 153 were considered to have a high QOL.

All forms were reviewed and any missing data were obtained. Data entry, processing and statistical analysis were carried out using SPSS 16.0 statistical software package. The chi-square test of significance was used to compare results from different groups. $P < 0.05$ was considered to be statistically significant. Correlation coefficient (person correlation) was used to measure correlation between quality of life score with age and social score.

3. Results

Table (1) shows the distribution of studied sample according to personal data of visually impaired child, Assiut City. It was found that more than two-thirds of studied children aged between 12-18 years. The mean age of studied children is 15.87 ± 3.03, more than half of them were male. The majority of studied children live inside the school (85%). As regards grades of education, less than one thirds of children were in the primary stage, less than half of them were in the preparatory stage and about one-third of them were in the secondary stage of education. Regarding birth order, the second and the third birth approximately equally order were represented in one quarter of the studied sample. As regards family size, about half of studied sample were from family sized 4-6 persons. According social class score more than half of studied children were male nearly three quarter of studied sample are from low social class (72%) and only (14%) of the studied sample high score.

Fig. (1) shows that distribution of studied sample regarding to causes of visual impaired Illustrates that among the overall causes of blindness, about three-quarters of studied children had a congenital cause of their disability

Table (2) shows the distribution of studied sample regarding to their total quality of life domains of visually impaired children. More than half of the studied children had low score of total quality of life regarding emotional status, social and personal relationships. As regarding physical health, more than half of studied sample (56%) had moderate score of total quality of life and two-thirds of them had moderate score regarding economical status. According to the environment domain, 68% of studied sample had high total quality of life score and 75% of them had high score regarding spirituality / religion and personal beliefs.

Fig. (2) shows that distribution of studied sample regarding to their total quality of life score of visually impaired children 70% of the studied sample had moderate total quality of life score.

Fig. (3) shows that distributions of studied sample regarding to their total Self-esteem score of visually impaired children nearly half of studied sample had normal Self-esteem (47%).

Table (3) shows that relation between total quality of life score and personal characteristics of visually impaired children. Regarding school grades, the majority of primary school children had moderate total quality of life score and no one of them had high score (P-value 0.000). As regards school residence, it was found that 40% of studied children with high total quality of life scores were outdoor school residence compared to only 8.2% of indoor school residence (P -value 0.003). According to sex, the majority of studied children with moderate total quality of life score were male compared to only 7%

with low total quality of life score. On the other hand, Female gender was represented in 58.1% with moderate total quality of life score compared to about one third of them with low score with statistically significant difference (P -value 0.009).

Table (4) shows that relation between total self-esteem score and personal characteristics of visually impaired children. Regarding self-esteem score, more than half of preparatory school children had low self-esteem score, while more than two thirds of secondary school children had normal self-esteem score. As regards school residence, about three quarters of outdoor school children had normal self-esteem score. According to the number of children in the family 71.4% of normal self-esteem children were present in small family compared by two thirds of low Self-esteem score were present in large family (more than 6 children) with no statistically significant difference

Fig. (4) shows correlation between age and quality of life score, the more the age increase, the more total quality of life score increase

Fig. (5) shows correlation between social and quality of life score, the more the social score increase, the more total quality of life score increase with highly statistically significant difference.

Table (5) show that relation between social class with total quality of life and self-esteem scores of visually impaired children It was noticed that nearly half of children with high total quality of life score were come from high social class compared by 8.3% from low social class children, with statistically significant difference. As regard self esteem and social class scores more than half of studied children with low social class had low self-esteem compared to more than three-quarters of normal self-esteem children were come from high social class but the differences were not statistically significant.

Table (1): Distribution of studied sample according to personal data of visually impaired child, Assiut City

Personal data	No. (n= 100)	%
Age: (years)		
12<18	69	69.0
18+	31	31.0
Mean ± SD	15.87 ± 3.03	
Sex:		
Male	57	57.0
Female	43	43.0
School residence:		
Indoor	85	85.0
Outdoor	15	15.0
Years of education:		
Primary (last two years)	22	22.0
Preparatory	44	44.0
Secondary	34	34.0
Birth's order:		
First	20	20.0
Second	25	25.0
Third	24	24.0
Fourth	12	12.0
Fifth and more	19	19.0
Family size:		
1-3	21	21.0
4-6	51	51.0
6+	28	28.0
Score of social class:		
Low	72	72%
Moderate	14	14%
High	14	14%

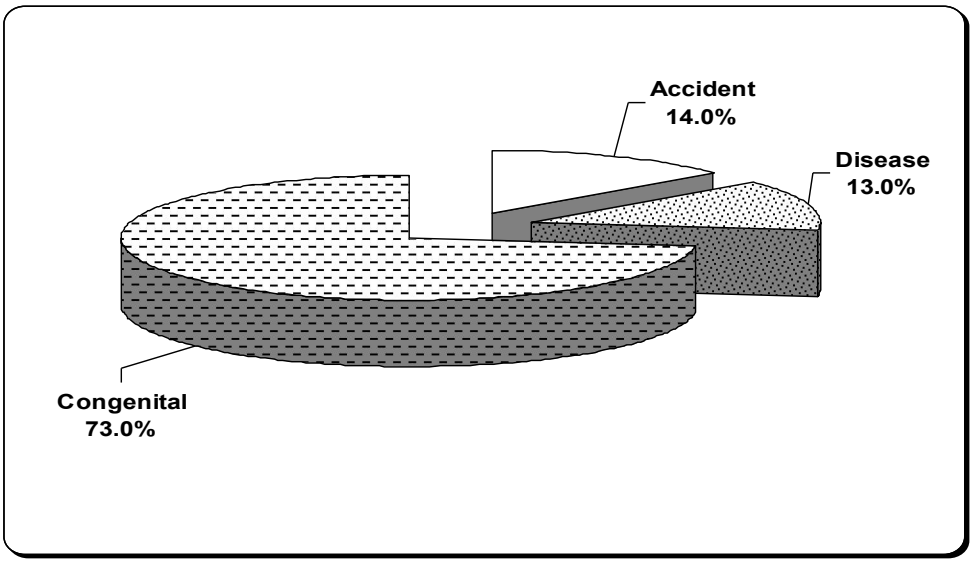


Fig. (1): Distribution of studied sample regarding to causes of visual impaired

Table (2): Distribution of studied sample regarding to their total quality of life domains of visually impaired children

Quality of Life Domains	Low (<50) (n= 17)		Moderate (50-<75) (n= 70)		High (>75) (n= 13)	
	No.	%	No.	%	No.	%
1. Physical Health	39	39	56	56	5	5
2. Self dependence	1	1	66	66	33	33
3. Emotional Status	59	59	24	24	17	17
4. Social and personal Relationships	52	52	32	32	16	16
5. Environment	4	4	28	28	68	68
6. Spirituality/ Religion/ Personal beliefs	8	8	17	17	75	75

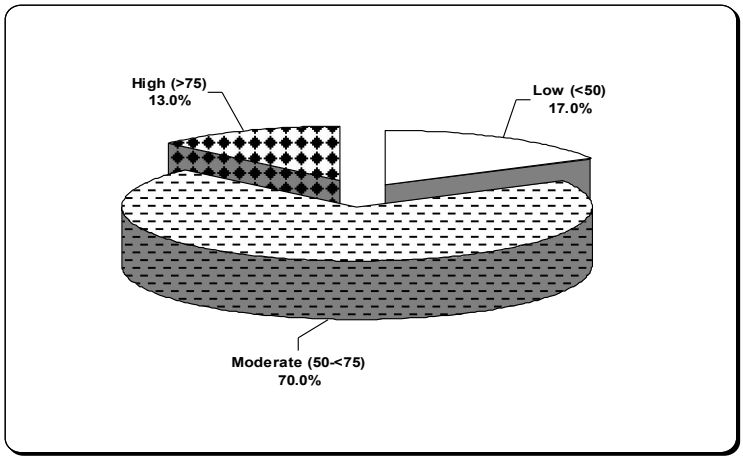


Fig. (2): Distribution of studied sample regarding to their total quality of life score of visually impaired children

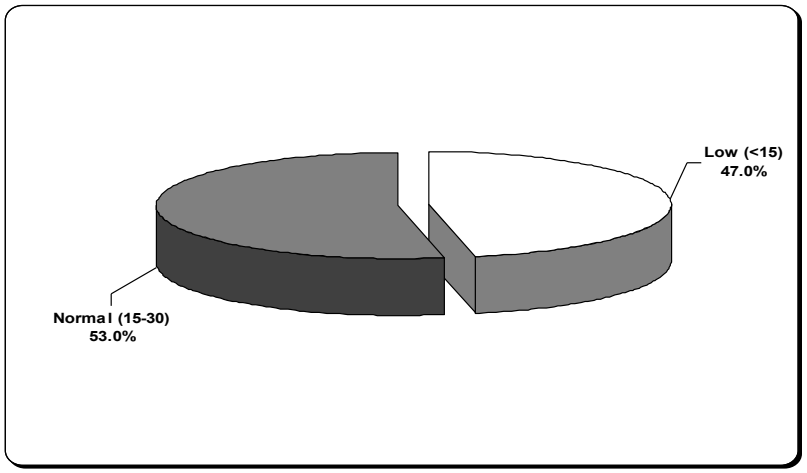
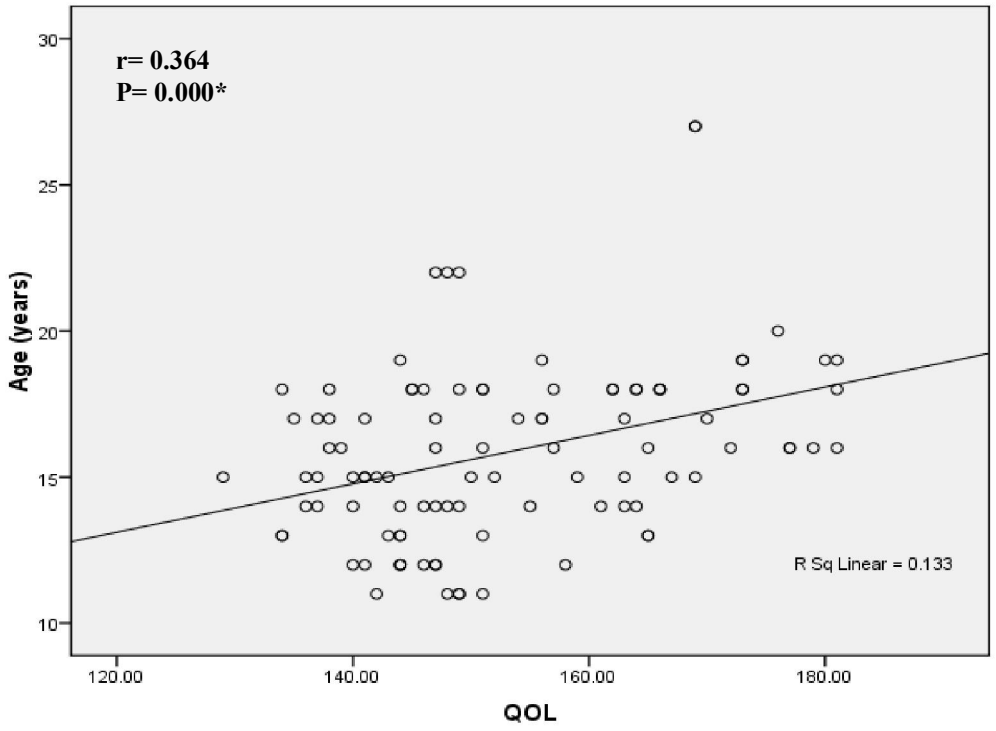


Fig. (3): Distribution of studied sample regarding to their total self-esteem score of visually impaired children

Table (3): Relation between total quality of life score and personal characteristics of visually impaired children

Personal characteristics	Total Quality of Life (n=100)						X^2 (P-value)
	Low (n= 17)		Moderate (n= 70)		High (n= 13)		
	No.	%	No.	%	No.	%	
School grades:							
Primary school	3	13.6	19	86.4	0	0.0	32.45 (0.000)*
Preparatory school	14	31.8	29	56.9	1	2.3	
Secondary school	0	0.0	22	64.7	12	35.3	
School residence:							11.41 (0.003)*
Indoor	15	17.6	63	74.1	7	8.2	
Outdoor	2	13.3	7	46.7	6	40	
Sex:							9.39 (0.009)*
Male	4	7	45	78.9	8	14	
Female	13	30.2	25	58.1	5	11.6	
Number of children in the family:							
1-3 children	5	23.8	12	57.1	4	19.0	2.89 (0.577)
4-6 children	8	15.7	36	70.6	7	13.7	
> 6 children	4	14.3	22	78.6	2	7.1	
Birth order:							
First	2	10	16	80	2	10	8.58 (0.379)
Second	5	20	15	60	5	20	
Third	7	29.2	14	58.3	3	12.5	
Fourth	2	16.7	8	66.6	2	16.7	
Fifth and more	1	5.3	17	89.5	1	5.3	
Father education:							
Illiterate/ read & write	7	21.9	20	62.5	5	15.6	5.17 (0.271)
Basic education	3	14.3	18	85.7	0	0.0	
Secondary/ university	7	14.9	32	68.1	8	17.0	
Mother education:							
Illiterate/ read & write	9	13.6	52	78.8	5	7.6	8.33 (0.080)
Basic education	3	27.3	6	54.5	2	18.2	
Secondary/ university	5	21.7	12	52.2	6	26.1	

Chi-square test * Statistical significant at P< 0.05


r= 0.364
P= 0.000*
Age (years)
30
25
20
15
10
R Sq Linear = 0.133
120.00
140.00
160.00
180.00
QOL

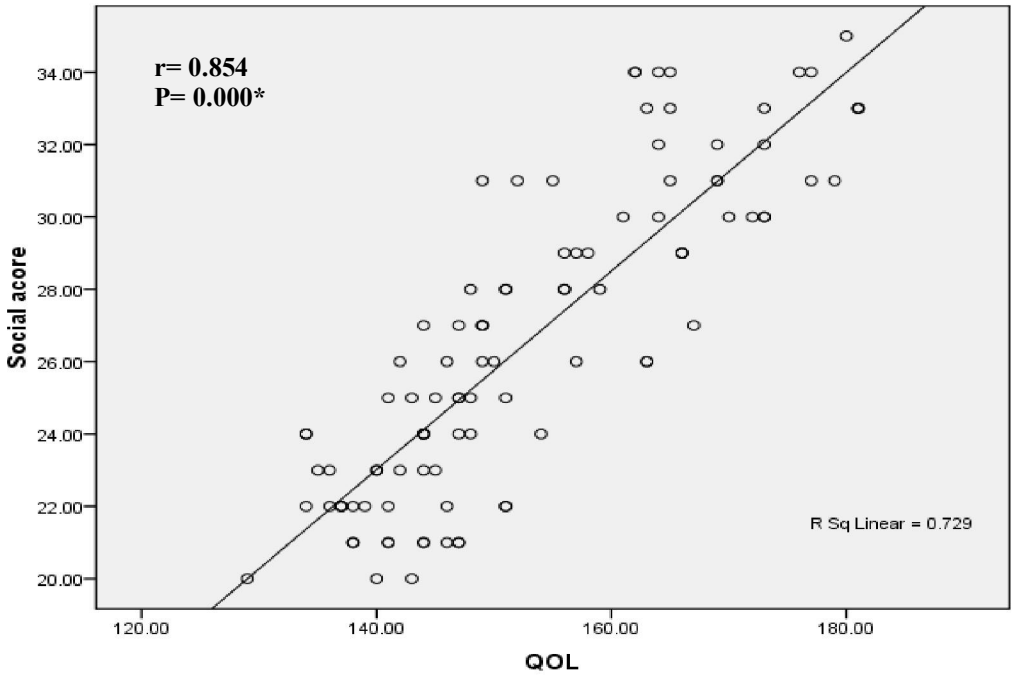

r= 0.854
P= 0.000*
Social acore
34.00
32.00
30.00
28.00
26.00
24.00
22.00
20.00
R Sq Linear = 0.729
120.00
140.00
160.00
180.00
QOL

Table (4): Relation between total Self-esteem score and personal characteristics of visually impaired children

Personal characteristics	Total Self-esteem score (n=100)				X^2 (P-value)
	Low (<15) (n= 47)		Normal (15-30) (n= 53)		
	No.	%	No.	%	
School grades:					3.59 (0.166)
Primary school	10	45.5	12	54.5	
Preparatory school	25	56.8	19	43.2	
Secondary school	12	35.3	22	64.7	
School residence:					2.93 (0.087)
Indoor	43	50.6	42	49.4	
Outdoor	4	26.7	11	73.3	
Sex:					1.69 (0.194)
Male	30	52.6	27	47.4	
Female	17	39.5	26	60.5	
Number of children in the family:					4.98 (0.083)
1-3 children	6	28.6	15	71.4	
4-6 children	24	47.1	27	52.9	
More than 6 children	17	60.7	11	39.3	
Birth order:					3.31 (0.508)
First	8	40	12	60	
Second	12	48	13	52	
Third	9	37.5	15	62.5	
Fourth	6	50	6	50	
Fifth and more	12	63.2	7	36.8	
Father education:					4.18 (0.124)
Illiterate/ read & write	18	56.3	14	43.8	
Basic education	12	57.1	9	42.9	
Secondary/ university	17	36.2	30	63.8	
Mother education:					8.08 (0.018)*
Illiterate/ read & write	35	53.0	31	47.0	
Basic education	7	63.6	4	36.4	
Secondary/ university	5	21.7	18	78.3	

Chi-square test * Statistical significant at P< 0.05

Table (5): Relation between social class with total quality of life and self-esteem of visually impaired children

	Social class						X^2 (P-value)
	Low (n= 72)		Moderate (n= 14)		High (n= 14)		
	No.	%	No.	%	No.	%	
Quality of life:							17.33 (0.002)*
Low	10	13.9	5	35.7	2	14.3	
Moderate	56	77.8	8	57.1	6	42.9	
High	6	8.3	1	7.1	6	42.9	
Self-esteem:							4.74 (0.094)
Low	38	52.8	6	42.9	3	21.4	
Normal	34	47.2	8	57.1	11	78.6	

Chi-square test * Statistical significant at P< 0.05

4. Discussion:

Blindness is a serious disability for the individual, his/her family and the community. WHO has always been conscious of the fact that blindness and visual disability are public health problems. However, for a long time, the magnitude of this problem could not be assessed and meaningful global prevention activities could not be initiated because of lack of epidemiological data and information. In this study, it was observed that more than half of visual impaired children were males (57.0%) and (43.0%) were females, these findings supported by Boulton, et al (2006) who found that 59.5% of the studied children were males. The same results were obtained in many studies conducted by Khallaf and Khalifa, (2004); Adegbehingbe et al., (2005); Reddy et al., (2008) and Shrestha et al. (2009) in which visual impairment are more prevalent among males. This problem may based on that males are exposed to environmental pollution, unhygienic health practice, infection and trauma inside- and outside home. On the other hand, Khandekar and Abdu-Helmi (2004); Adegbehingbe et al. (2005) and El-Moselhy et al. (2005b) found that vision impairments more common among females.

Blindness can occur as a result of a number of infectious and non-communicable diseases, as well as injuries. In this study, congenital causes accounted for 73% of causes of blindness. This is in agreement with Kamel (1987) who reported that the main causes of blindness among blind students were congenital anomalies, followed by infection, then trauma and also with El-Gilany et al (2002) who commented that congenital causes accounted for 48.7% of causes of blindness.

Regarding the child's age, our findings constituted more than tow-third of the studied children were in the age group 12 -18 years, this is in agreement with El-Gilany et al (2002) who represented that about 71.7% of the study sample were in age group 10-19 year. Also, Khandekar and Abdu-Helmi (2004) found that rate of vision impairment was significantly higher among high age group 12- 16 years ($P<0.0001$).

As regard socioeconomic risk factors, poverty is a major factor contributing to blindness and visual impairments (The USAID Child Blindness Program, 2007). According to findings in this study, the majority of studied children had low social class (72%), which in agreement with El Gilany et al., (2002) who found that 79.8% of studied sample participant among low and very low social class. Similar finding have been reached by Ajaiyeoba and Scott (2002) who found that (73.0%) of visual impairment children in Nigeria were from the lower socioeconomic class. Also, in India the large number of blind children among the rural population was from the lower socioeconomic class (Jain et al., 2005). In contrast with the foregoing results, Boulton et al (2006) reported that there is no significant difference in visual impaired children by socioeconomic classes.

According to total quality of life domains among visual impaired children, results of the present study clarified, the highest percentage of them had high score of the spirituality /religion and personal beliefs domain and environment domain. Regarding the spirituality /religion and personal beliefs domain, 75% of studied sample had high score. This finding in agreement with Abo El-Magd (2001) who found that the most studied sample had higher mean score of total quality of life related spiritual domain .This could be explained by the fact that society norms in our culture gives special concern to teaching the young facts and behaviors related to their religion a matter which enriches the adolescent spiritual life and attitude. Also, 68% of them had high score of environment domain, similar finding were revealed in Fahmy et al (2010). Conversely, the finding of the present study revealed that more than half of studied sample had low score of social and personal relationships domains. These findings are quite close to those of El-Gilany et al (2002) who found that 44.2% of studied sample their social role had affected by blindness, also have reported that the major impact of blindness was on relations with friends.

The present study revealed also that nearly half of children with high total quality of life score were come from high social class. This finding in agreement with Mohamed (2002) who reported that studied sample with high income had higher mean score of total quality of life than those who had lower income.

Concerning sex as a factor in quality of life, results showed that the majority of studied children with moderate total quality of life score were male compared to only 7% with low total quality of life score. This finding in agreement with Mohamed (2002) who reported that the quality of life scale being higher among male than female, This observation may be explained according to our Egyptian culture by the fact that families usually give more attention to their sons than daughters, so male receive better medical and psychological care than females. Furthermore age as a factor in quality of life; results revealed that there is an equable relation between age and quality of life. Similar finding have been reached by Mohamed et al (2009) who reported that more the age increase the more quality of life score increase.

Regarding mother education, the present study noticed a positive correlation between high education

of mother and normal self-esteem of visual impaired children. There is possible explanation for this finding, the mother's education play an important role in the understanding the children needs and feeling and increase their self confidence and make him move toward an independent life and increase their self confidence.

The finding of the present study indicated that about half of the studied children had normal self-esteem, this finding in contrast with El Gilany et al. (2002) who found that most of the sample had low self-esteem.

Regarding quality of life and self-esteem, there are high score among children lives with their family (outdoor school residence) (40% and 73.3% , respectively). There are several possible explanations for these findings, all based on families may support the children socially, financially and provide them a suitable places for living in the home more than those levies inside the school. As regards social class, more than three quarter of studied children of this study with normal self-esteem (78.6) had high social class.

According to the findings of the present study, 71.4% of studied children had normal self-esteem were present in small number family compared to more than two third of low self-esteem present in large number family. The rationale for these findings is the parent in small family are more contact with their children and provide them a lot of care, security and attention.

Conclusions:

It is concluded that children from high social class had higher quality of life and normal self-esteem than other children.

Recommendations:

It is recommended that:

Multi-component intervention with a focus on the children and their families are needed to improve self-esteem and quality of life of visually impaired children and school health program.

Designing and implementing of a psychological counseling program for visually impaired children to improve their emotional status and help them to coping effectively.

School health program for teachers and caregiver about ideas that can be implemented and behaviors that should be adopted by those in contact with visual impairment children, to giving them guidance needed to develop a full potential for autonomous community living.

Future follow-up studies with larger number of sample size are needed to determine the impact of visually impaired on quality of life of visual impairment children

It is better that educational institution (Noor School) to construct consulting centers besides school's for accepting blind and disabled children, to consult parents about problems due to child's disability and other problems.

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A Newly Developed Exercise Program for Treatment of Mechanical Low Back Pain Associated with Accentuated Lumbar Lordosis

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Abstract: The relationship between low back pain and lumbar lordosis has been studied extensively with many contradictory conclusions. The already existing exercise programs to correct the accentuated lumbar lordosis have got many disadvantages. Objectives: Introduce newly developed exercise program to verify the relationship between mechanical low back pain and increased lumbar curve. A new program of exercise has been designed to alleviate the disadvantage of the previous programs. Study design: experimental, one group . Twelve patients with average age 39.3±4.5 years were included in the study, all have chronic low back pain over one year with lordotic angle over 50 degrees. Lateral X ray-films were taken before, after one and two months of treatment .A metal frame was designed to facilitate the use of plumb line, also a wedged pillow with an angle of 115˚ and stretch tool was designed for the execution of the exercise program. The patients did not receive any source of heat or medication throughout the treatment. The results revealed to a significant reduction of pelvic inclination, lumbosacral angle and lumbar curve were 5.4˚, 2.6˚ and 3.00˚ , respectively after the end of the second month of treatment . There is gradual and complete relief of pain without recurrence. It is concluded that this new program alleviated the disadvantages of the previous programs .It confirm the relation between low back pain and accentuated lumbar lordosis.
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Keywords: lordosis, lumbosacral angle, pelvic inclination, X-ray measurements, accentuated, correction, William's exercises, mechanical low back pain

1. Introduction:

Mechanical causes are responsible for the greatest percentage of low back pain diagnosis. It does not matter which tissue is involved in the mechanical low back pain but rather the abnormal or excessive forces that converge into the region and result in pain[1].The change in degree of lumbar lordosis have been identified by some investigators as the main cause of low back pain while other found no relationship between the degree of lumbar lordosis and low back pain. From the mechanical point of view the accentuated lumbar lordosis is associated with an increased prevalence of low back pain[2] , an imbalance in trunk muscle strength can influence significantly lordotic curve of lumbar spine and might be one risk factor for potential LBP[3]. No significant difference between normal subject and those with low back pain concerning the degree of lumbar lordosis. High and low value of lumbar lordosis was found in both groups [4,5]**.** No difference in lordosis between those affected with lumbar spine osteoarthritis and those who are disease free[6]**.**

In standing posture, a change in pelvic tilt either anterior or posterior, produces an almost equal angular change in lumbar lordosis, the degree of lumbar lordosis change significantly during the time period of the exercise. No evidence suggests that these exercises will promote a permanent change in posture[7]. Youdas[8] found no relationship between the level of pelvic inclination and the degree of lumbar lordosis in a standing position. There is a weak association between lumbar lordosis and pelvic inclination, but the abdominal muscle force of chronic LBP subject was less than that of the control subject[9]. A strong correlation between pelvic tilt angle and thoracic kyphosis and lordosis found by Legaye and Beapere[10] who assessed the onset of spinal deviation with pelvic inclination. Variations in pelvic morphology may significantly influence measures of pelvic tilt and innominate rotational asymmetry[11].

William's exercise program was developed in 1937 for patient with chronic low back pain for men under 50 and women under 40years who had exaggerated lordosis of lumbosacral part of the spine [12] .No x-ray study was found to proof the reduction of lumbar lordosis after using William's flexion exercise, instead the program faced great criticism. The experimental study don by **Rici, *et al.*[13]** revealed that during the sit up exercise, the pelvis was found to be tilted and put the spine in hyperextension before forward trunk displacement. **Jackson and brown [14]** believed that the exercise to stretch the low back muscle is not necessary as the low back muscles are inter segmental and don't have the mechanical advantage to increase lumbar lordosis.

Kendal and Jenkens[15] mentioned that the isometric exercises using valsalva maneuver were particularly beneficial for patient with prominent lumbar lordosis. This claim was denied by Davise *et al.*[16] .They found no significant alteration of the mean depth of lordosis after 4weaks of treatment using the isometric flexion exercise. The valsalva maneuver did raise intra-abdominal pressure, however it increased rather than decreased spinal load. Press heel sit up increase psoas muscle activity[17].Bent knee sit up may be problematic exercise for people with LBP pathologies because of the relatively high rectus femoris and lumbar paraspinal muscle activity. When these muscles contract, the force generated act to anteriorly rotate the pelvis and increase the lordotic curve of the lumbar spine[18.]

Proper reduction of lumbar lordosis helps to support load on the ligamentous system .This can be accomplished by rotating the pelvis posteriorly and by proper instruction to use abdominal muscles without necessity to bend forward. The thoracolumbar fascia is the only active ligamentous structure capable of balancing the external moment .It is under muscular control through the entire range of spinal motion, whereas the midline ligament is geometry dependant [19]. Contraction of the latissimus dorsi , internal oblique or transverses abdominis can exert active tensile force to the thoracolumbar fascia. Maximizing the effectiveness of the thoracolumbar fascia can be accomplished by training muscles attached to it and utilization of its passive mechanism[1]. Vleeming *et al.*[20] revealed that the fascia attached to the latissimus dorsi is continuous with the fascia of the gluteus maximus in the contra-lateral site. The gluteus maximus and contralateral latissimus dorsi muscle tense the posterior layer of thoracolumbar fascia and provide a force closure perpendicular to sacroiliac joint. The overall trend of latissimus dorsi muscle is to complement erector spine muscle in that when activity of one increased the activity of the other is decreased[21].

Fairweather and Sideway[22] used an adaptive form of idiokinetic imagery that incorporate kinesthetic awareness exercises that improve posture and reduce low back pain. Mental practice may prove to be a useful adjunct to traditional treatment options aimed at increasing muscle strength[23]. Kinesthetic imagery implies somesthetic sensation elicited by action[24]. The purpose of this study is to introduce newly developed exercise program to reduce the pain associated with accentuated lumbar curve, and verify the relationship between mechanical low back pain and increased lumbar curve. It included strengthening and stretching exercises based on biomechanics of the spine and muscle action, the key exercise is posterior pelvic tilt executed through ideokinetic imagery exercise.

2. Methods

Subjects

The study group included 12 patients (8 females and 4 males). Patients were adult with average age 39.3± 4.5 years, weight (81.66± 12.8 kg) and height (161.75±6.9 cm). Total Nineteen patients with chronic low back pain selected consequently from the out patient clinic with age ranging 30- 45 years. They were clinically examined by orthopedist then referred to department of radiology. They were complaining of back pain in spite of previous methods of treatment, including bed rest, pain killer, physiotherapy modalities and acupuncture. They complained of low back pain which was continuous for more than one year. The study included only the twelve patients who had lumbar lordosis greater than 50 degrees measured on x ray. Subjects were excluded if they had nerve root pain, neurologic sign and symptoms, previous spinal surgery or structural deformities such as scoliosis or spondylolisthesis. The study has been don in the Orthopaedic clinic, departments of radiology and physical therapy of Elharm Hospital, Giza Egypt. Informed consent was obtained and the right of subjects were protected.

X ray measurements

Lateral x-ray films were taken from standing position. The plumb line was used to standardize the technique of measurement. A metal frame was used to facilitate the use of plumb line. It is a movable plumb of metal wire. It was selected carefully to be not distorted to help in taking an accurate measurement. The plumb line represents the line of gravity on the external surface of the body. The x-ray was repeated after one and two months of starting treatment. The distance of the x ray tube to the film was fixed by one meter. The x-ray films were taken by the same technician who was aware of necessity for consistency in technique. The x-ray measurements were the degree of lumbar lordosis (L_2-S_1) using standard cob technique, the upper border is a line parallel to the upper surface of the second lumbar vertebrae while the lower border is a line parallel to the upper border of the sacrum .The lumbosacral angle is the angle between the lower border of L_5 and upper border of S_1, pelvic inclination is the angle between the vertical line and the anterior surface of S_1 (Fig. 3). Cases with a lumbar lordosis greater than 50 degrees were only included in the study.

Designed tools

The designed wedged pillow is made of compressed sponge, it has an angle of 115 and nearly square base with dimensions of 65x 50cm, two separate bases of 4cm each were made to modify the height of the pillow according to the height of the patient. The designed pillow helps to keep the knee bent with an angle of 65°; this design was made according to electromyographic finding by **Walter and Partridge**[5] who found that hip flexors are fairly relaxed and participate to a lesser degree when the knee flexion was 65° instead of 90°. (Fig. 1 A). The stretch tool was designed to stretch hip flexors .The main idea of the tool is to design a tool with a changeable angle started from5-30 degrees with 5degrees interval. The general form and solidity were taken in consideration (Fig. 2 F).

Procedures

Pain level was measured with visual analogue scale. The scale of pain has an extreme point representing maximum pain while the other end represents no pain, The scale of pain was then portioned into 10 sections with 0 indicating no pain and 10 indicating maximum pain. Each patient was asked to identify the point appropriate to their level of pain. This identification was recorded at the end of each weak of treatment.

The newly developed exercise program included strengthening and stretching exercises .The strengthening exercises were posterior pelvic tilt exercise implemented through idiokinetic imagery exercise, posterior pelvic tilt with hips abducted and externally rotated ,curl up exercise by raising the head then gradually to elevate the scapula off the plinth, latissimus dorsi exercise to train the transverse and oblique fibers of latissimus dorsi which attached to the thoracolumbar fascia. Shoulder lift trunk rotation, gluteal setting exercise and hip extension exercise.

The mechanical basis of the exercise program is to develop an active tension in the thoracolumbar fascia by training the muscles attached to it. The thoracolumbar fascia will be capable of sub serving antiflexion role complementing that of the back muscles. This mechanism will help to decrease the activity of back muscles (longissimus and iliocostalis of thoracic part of erector spine and multifidus). All the exercises were executed from lying position with upper trunk stabilized to minimize the activity of paraspinal muscles. The stretch tool was used to provide low load prolonged stretch. The exercises preceded stretching because the increase in tissue temperature Produced by muscular exercise allows elongation to occur with less structural damage. The stretching exercise is followed by strengthening exercise for the gluteal muscle. The exercise program is preceded by Relaxation and weight exercises. Figure 2 show most of the exercise program.

Weight exercise: patient was made aware of two objects and their respective weight. Two sandbags (1 and 1.25kg) were placed 10 seconds for each with 5 seconds interval on the abdominal area. The purpose of the weight exercise is to increase awareness of the abdominal area and hence facilitate effectiveness of idiokinetic imagery exercises.

The first exercise (posterior pelvic tilt) executed through the idiokinetic imagery exercises. It helps to train the abdominal muscles without including back mscles, so avoid co contraction which caused by pressing back and holding breath. Each exercise was repeated precisely three sets of ten with rest in between .Only a new exercise was added each sitting, however the patient performed each exercise correctly before preceding to a more complex one. The treatment was given three times/week. The time allowed for the exercise program was gradually increased in parallel with the increase of the exercises; it is started with half an hour to reach 1.5 hours for full program.

Exercises in the first month of treatment.

Strengthening exercises

The following exercises were given in order from the hook lying position. The knees were bent at an angle 65° degrees .this position was maintained passively through using the designed wedged pillow.

1-Posterior pelvic tilt

Too often the posterior pelvic tilt is done without any benefit of the abdominal. The subject performs the movement by pushing the feet to help rock the pelvis back into posterior tilt [26] .Caillet[2] mentioned that the movement of posterior pelvic tilt is performed by the combined contraction of the abdominal and gluteal muscles, pressing the low back against the floor. This method may cause valsalva maneuver which increase the activity of the back muscles. In the current study posterior pelvic tilt was implemented through the following idiokinetic exercises (Fig 1 A). Various facilitation techniques were uses throughout the program consisted of multisensory (auditory, tactile, visual, and kinesthetic cuing).

A-Patient imagine his abdomen like a hand pulling the weight on the abdomen downward towards the plinth.

B-Patient imagines his trunk as a flexible curve like the flexible rule and then gradually slid it downward to be straightened on the plinth. The therapist demonstrates this to the patient using a flexible rule.

C-Patient imagine each side of his pelvis as a wheel, both wheels revolving anticlockwise toward the head. After executing each component of posterior tilt correctly, patient then performed the previous exercises as one exercise.

Mechanism of muscle action

Idiokinetic imagery exercises help to perform the exercise under consciousness and to be executed slowly and precisely without co –contraction, they were a modification of that used by fair-weather and sideway[22],they were modified according to the understanding of patients and their ability to imagine.

Posterior pelvic tilt requires moderate activity of internal and external oblique muscles, this helps to generate intra-abdominal pressure [14],it helps to stretch the long fascicles of multifidus , only the long fascicles of multifidus has the capability to increase lumbar lordosis. It also helps to train the longissimus and iliocostalis muscles of the lumbar erector spinae, these muscles has the ability to decrease lumbar curve by inducing posterior shear[27]**.**It Allows the patient to adjust his lordosis to support load through ligamentous tension[28]**.**Posterior pelvic tilt ,not only preceded the other exercises each sitting but it was a component of them. This helps to return to the normal pattern of the abdominal muscles and increase the repetition of the posterior pelvic tilt which is the key exercise for correcting lumbar curve.

2-Posterior pelvic tilt with hip abducted and externally rotated with heels together.

This exercise helps to regain maximus performance of gluteus maximus from the crock lying position.

3-Curl up exercise

The patient performed posterior pelvic tilt then curled the trunk. He raised the head then progressed by raising the shoulders .The patient raised himself enough to elevate the scapula, then return back slowly while maintaining pelvic tilt(Fig. 1 B)

Mechanism of muscle action

This exercise produce minimal amount of lumbar flexion and provided maximum activity of external and rectus abdominis muscle as confirmed by halpern and bleck[29].It provides the advantage of exercising the abdominal muscles without strong hip flexor exercise .In addition there is less intra- discal pressure when doing trunk curl as compared to completing the sit up. The inferior and medial direction of the external abdominal oblique muscles Help to posteriorly rotate the pelvis and check the downward and forward motion of the pelvis.

4-Shoulder lift trunk rotation

The patient tilt the pelvis posteriorly then elevated one shoulder and rotated the shoulder on the pelvis (Fig. 1 C)

Mechanism of muscle action

Trunk rotation performed largely by internal oblique [25].Training the Internal oblique helps to generate intra abdominal pressure reflexly while the deliberate increase in inta-abdominal pressure using valsalva maneuver cause increase in erector spinae activity .This exercise add tension to the thoracolumbar fascia. It increases the force of the extension moment that the fascia can generate. This will help back muscles in overcoming the large flexion moment and consequently decrease the activity of paraspinal muscle.

5-Latissimus dorsi exercise

The patient tilt the pelvis posteriorly then adducting the arm against resistance (Fig. 1 D)

Mechanism of muscle action

The latissimus dorsi muscle has the largest moment arm length; it potentially influences lumbopelvic mechanics with less effort. It has significant effect on lumbopelvic mechanics through its attachment to the thoracolumbar fascia. This exercise was designed to train the part of the latissimus dorsi which attached to the thoracolumbar fascia. It avoid involvement of the direct part of the muscle which constitute the most lateral part (2-3cm).This part is ultimately attached to the iliac crest and its training will cause an increase in the anterior pelvic tilt .It causes bilateral pull on the thoracolumbar fascia by the transverse and oblique fibers of both latissimus dorsi, this help to develop an active tension in the thoracolumbar fascia which ensheaths the back muscles .The tension developed in the fascia helps to brace the erector spinae preventing their dorsal displacement, hence they can work with less effort .The latissimus dorsi muscle complement the erector spinae, therefore increase the activity of latissimus dorsi through training will help to decrease activity of erector spinae and consequently help to reduce lordotic curve. The exercise was preceded by posterior pelvic tilt. The patient maintained this position then adducted against resistance. This exercise allows coupled function of the gluteus maximus and contralateral latissimus dorsi. The gluteus maximus and contralateral latissimus dorsi muscle tense the posterior layer of thoracolumbar fascia. They provide a pathway for uninterrupted mechanical transmission between pelvis and trunk. This coupled function creates a force perpendicular for the sacroiliac joint .The thoracolumbar fascia

exerts compression on the lower lumbar spinae and pelvis, therefore ,it helps to accomplish force closure of SI joint physiologically instead of using pelvic belt.

Stretching exercise

The stretching exercises started on the 4th sitting and preceded by the previous strengthening exercises The starting position for stretch helps to rotate pelvis posteriorly(Fig. 1 E), this implies a decreased risk of hyperextension of lumbar spine .The more posteriorly tilted pelvis in this position means greater extension of the hip[21].The starting position of stretch also helped the stretch to begin when the muscle was in completely relaxed state to minimize the amount in tension developed by the contractile component . A cushion put under the abdomen ,the pelvis was stabilized by designed belt to reduce the magnitude of electrical activity in the sacrospinalis following the study of **Fisher and Houlz [30]** .The starting position was maintained for 5 minutes, then the flexed leg was raised to the bench for one minute .From the starting position the limb was supported on the designed stretch tool(Fig. 1 F) for five minutes elevated 5degrees then lowered for one minute rest in the prone position then elevated another 5 degrees for another five minutes. The stretch was repeated for the other leg. This procedure allows the stretch to be maintained 5 minutes in each new length of hip flexor. The designed stretch tool helps to provide low force long duration stretch technique. The stretching exercise was followed by gluteal setting exercise from prone position.

Gluteal setting exercise

The stretching exercise was followed by gluteal setting exercise. Strong volitional contraction was performed from prone position with cushion under the abdomen.

Mechanism of muscle action.

In the presence of tight hip flexor, an attempt to strength hip extensors might therefore result in overuse of perpetuating tightness of the paraspinalis. Increased erector spinae activity may be seen in the gluteus maximus weakness, the lift is accomplished through a forward pelvic tilt and hyperextension of the lumbar spine resulting in posterior compression and anterior shear in the lumbar region. So initial attention was focused on gluteal setting which elicited a large degree of activity regardless of the position.

The exercises in the second month

All the exercises were performed every sitting, the stretching exercise was modified, In the second month the stretch was maintained with the stretch tool at 10 degrees for 5 minutes repeated three times.

Hip extension with abduction and external rotation

From the starting position of the stretch, strengthening for hip extensor was performed after stretch. The starting position of this exercise was the stretch position but the extended limb was put in abduction and external rotation. The patient was asked to hyperextend the thigh.

Mechanism of muscle action

This exercise elicited the strongest contraction of the gluteus maximus muscle .Hip extension was accompanied with knee extension, so almost all rectus femoris activity disappeared as well as no activity in the hamstring. In this exercise the knee was extended, this help to train quadriceps. Contraction of the quadriceps causes tightness of the iliotibial band, half of the gluteus maximus attached to the iliotibial band. Large portion of the gluteus maximus act with greater leverage when the iliotibial band is tight .The exercise allowed extension beyond the Zero hip extension .It is associated with strong contraction of the gluteus maximus, while extension of the flexed thigh is performed primarily by the action of the hamstring muscle.

Data Analysis

The student t test and correlation coefficient were used to judge statistical significance difference. The level of significance was $p<0.05$. Data were analyzed using SPSS program version 12.0

3. Results

The differences in the three angles, lumbar curve, lumbosacral angle and pelvic inclination after the 1st and 2nd month of treatment were represented in three lateral x-ray films. They were taken for each patient before, after one month and finally after two months of treatment (Fig. 3). The result of the study showed a significant reduction in lumbar curve, lumbosacral angle and pelvic inclination, after the first and second month of treatment Table (1).The differences in lumbar curve were1.25±2.0 and 3.00±2.48 after the 1st and second month of treatment respectively. The differences in lumbosacral angle were 1.08±1.44and 2.66±1.87 after the 1st and 2nd month of treatment, respectively .The significant changes in pelvic inclination were2.08±2.87 and 5.41±1.78, respectively.

Fig. 1 The designed wedged pillow made of compressed sponge, with an angle of 115°

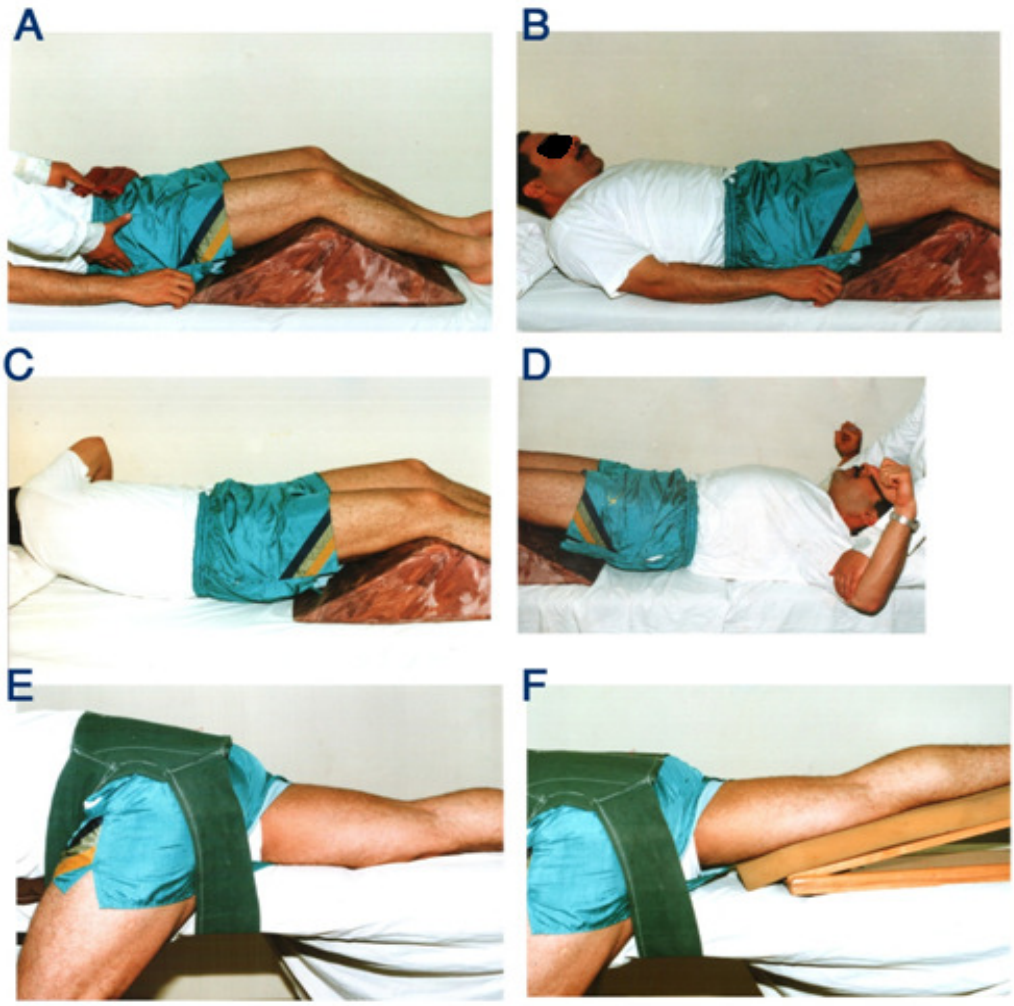
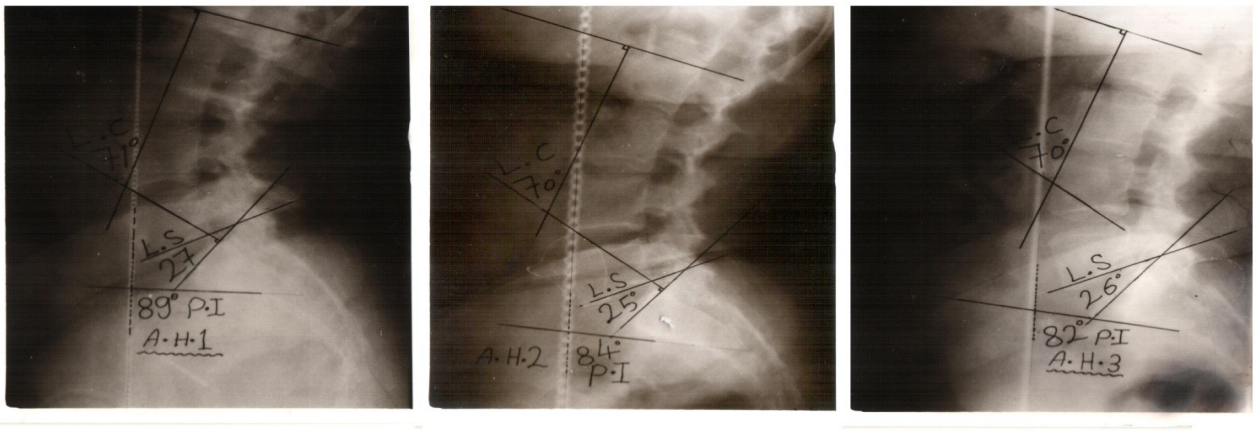


Fig. 2: Part of Newly developed exercise program .A .show posterior pelvic tilt through idiokinetic imagery exercise. B. Curl up exercise preceded by posterior pelvic tilt. C trunk rotation preceded by posterior pelvic tilt. D. Latissimus dorsi exercise for the transverse and oblique fibers preceded by posterior pelvic tilt. E, starting position of low load stretch where the pelvis stabilized by belt. F, low load prolonged stretch using designed stretch tool.



"FIGURE 3" X ray measurements pre treatment, after one month and 2 month of treatment, from left to right respectively.
PI: The angle of pelvic inclination **LS: lumbosacral angle**
LC: Angle of lumbar curve **A.H: The abreviation of patient's name**

There was a gradual decline of pain after treatment without recurrence. The pain was reduced by about50% at the end of the 3rd week and completely relieved by the end of the 8th week(Fig. 4)There was a correlation of the change in pelvic inclination with that of lumbar curve after the first month and second month equal to .8 and .513, respectively. The correlation is highly significant only after the first month of treatment where P=.002.

Table 1: Measurements of lumbar curve , lumbosacral angle and pelvic inclination before, one and two months after treatment.

X-ray measurements		Mean	T	Sig	Difference
Lumber curve	lumber curve before treatment	61.±5.79			
	lumber curve after 1 month	60.25±6.39	2.159	0.054	1.250
	lumber curve after 2 month	58.5±7.166	4.180	0.002	3.00
Lumbosacral angle	Lumbo-sacral angle before treatment	15.75±6.63			
	Lumbo-sacral angle after 1 month	14.6667±6.4	2.600	0.025	1.08
	Lumbo-sacral angle after 2 month	13.08±6.639	4.927	0.000	2.66
Pelvic inclination	Pelvic inclination before treatment	73.667±7.389			
	Pelvic inclination after 1 month	71.58±6.4	2.510	0.029	2.08
	Pelvic inclination after 2 month	68.25±6.689	10.532	0.000	5.416

Table 2: Correlation of the change of pelvic inclination with change in lumbosacral angle and lumbar curve.

Variables	Correlation after one-month	Correlation after two-month
Lumbar curve & Pelvic inclination	r =0.8 P.002**	r= 0.513 P.088

**Correlation is significant at the 0.01 level (2- tailed)

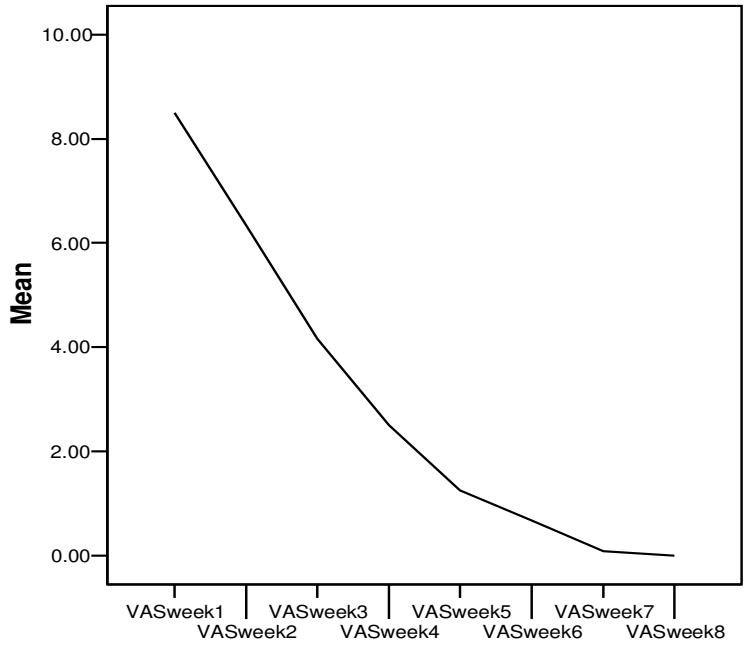


Fig. 4: Visual analogue scale of pain show complete relief of pain without recurrence by the end of treatment.

4. Discussion

This study concerned with applying a newly developed exercise program. It helped to correct the accentuated lumbar lordosis and decrease low back pain. The traditional exercise program of **Williams**[12] and isometric exercises[15] were believed to decrease lumbar lordosis however the biomechanical analysis[14],the experimental study by **Ricci *et al.*** [13] and electromyographic study by **Black Burn and Porteny**[31] revealed their disadvantages and the result of the few researches using such programs did not show any improvement in the lumbar curve[16,22].This study confirmed the relation between low back pain and accentuated lumbar lordosis , in contrast with **Youdas** [9] who mentioned that patients with CLBP had no more standing lumbar lordosis or pelvic inclination than their counterparts with healthy backs. **Nourbakhsh, *et al.*** [5] found no significant relationship between LBP occurrence and the degree of lumbar lordosis and concluded that the degree of lumbar lordosis did not differ between normal subjects and those with low back pain. This can be explained by the wide variation of the degree of lumbar lordosis among normal subjects [4,6] and any changes that might occur sooner or later may still within this normal range [6] and that large variation of pelvic morphology masks any difference in pelvic tilt between normal subject and a group of LBP[11]

In the present study the difference in the change of pelvic inclination is greater compared to the mean differences in the lumbosacral angle and lumbar curve, they were 5.41, 2.66 and 3.0, respectively after the second month of treatment. The results in the current study verified the suggestion of fair-weather and sideway [22] that the change in subject's lordotic angle was the result of reduction in anterior pelvic tilt and realignment of the spine as a whole. This in agreement with Day, etal[32] who mentioned that the lumbar curve can be altered by pelvic tilt. Anterior tilt increased the depth of lumbar curve and posterior tilt decreased the depth of lumbar curve, however his result was only at the time of exercise. A highly significant correlation in the current study was found in the change of pelvic inclination with that of lumbar lordosis after the first month of treatment. There was a significant reduction in pain in low back accompanied reduction in lumbar curve. In contrast to the study done by **Davis, *et al.*** [16] where the pain was reduced without change in lumbar curve. Our results goes with fair-weather and sideway[22] regarding the complete

disappearance of pain by the end of 8th week of treatment , however they mentioned that the cessation of pain had been preceded by initial sharp increase in pain level during the first week. In the present study, there is gradual decrease in pain without recurrence.

The hip flexor activity is less in the crunch than the knee bent set up[33] as the relative high rectus femoris muscle activity obtained with bent knee set up may be problematic for some people with LBP problem[18] .However, the curl up exercise executed in the current program is better than the crunch) .The designed billow allow the knee bent of 65 instead 90, this allow the rectus femoris to be fairly relaxed, also it avoid support foot on the plinth. In the current study the patient raised him self only enough to elevate the scapula. Following the study of **Halpern and** Bleck[29], this exercise produced minimal amount of lumbar flexion and provide maximum activity of external oblique and rectus abdominal muscles. In the current study Curl up exercise is preceded by maintaining pull in abdomen similar to Crunch exercise which show high EMG activity from the internal oblique which may offer more effective stabilization to the spine and pelvis[18] . Activation of the deep lateral abdominal muscles could be enhanced with proper instruction during the abdominal crunch, such as performing the abdominal crunch while holding the abdominal drawing in maneuver[34] .

In subject with normal abdominal muscles, as trunk flexion is slowly initiated by raising the head and shoulders from a supine position, the pelvis tilts posteriorly simultaneously[26] ; hence in this study every strengthening exercise was preceded by posterior pelvic tilt to return to the normal pattern of the abdominal muscles. In addition posterior pelvic tilt from hook lying position requires moderate activity of internal and external oblique muscles with minimal rectus abdominis activity, this help to develop intra abdominal pressure[14] . The increased lumbar lordosis increases the activity of the multifidus[28] , only the long fascicles has the capability to increase lumbar lordosis ,a reduction of the activity of the multifidus can be accomplished by posterior tilt exercise. It helps to stretch the long fascicles of multifidus. Posterior tilt exercise also helps to train the longisimus and iliocostalis muscles of the lumbar erector spinae, these muscles have the ability to decrease lumbar curve by inducing posterior shear[27]. Posterior pelvic tilt has been advocated to cause co-contraction of the local stabilization musculature and that for the posterior tilt to be performed the individual contract the lower abdominal muscles to rotate the pelvis posteriorly, so that the lumbar spines flatten out[35]. The contraction of the abdominal muscles accompanied the posterior tilt in the current study was implemented through ideokinetic imagery exercises. It is a method used to facilitate positive change in the spinal column of the subject through subcortical stimulation[22]. Slow eccentric curl-back phase and multisensory kinesthetic cuing without foot stabilization helped to increase voluntary recruitment of the internal oblique and transverses abdominis muscles, teaches abdominal muscle awareness and voluntary control to break the patterns of dependence on the iliopsoas muscles in exercise and functional trunk movement[36].

Some Commercial abdominal exercise devises have come to the market and have come popular with the public .A comparative electromyographic activity analysis between commercial abdominal exercise devices and conventional crunch start curl up revealed that the conventional curl up exercise is safer with less hip flexor activity[37] . Therefore, the technique used to execute Curl up ex in the current study is better than Some commercial abdominal ex (AbDoer'Ab twister,A b Roker and SAM) which recorded the lowest amount of abdominal activity and exhibit the greatest rectus femoris activity. Purchasing these abdominal devices does not appear to offer any advantage in recruiting abdominal musculature over performing traditional exercises that require no additional equipment[33] .The relative high rectus femoris muscle activity obtained with power wheel exercise may be problematic for some people with LBP problem[18] .While the makers of the Bodyblade claim that it is "the most efficient core power training tool ever designed". Use of the Bodyblade may either enhance or compromise spine stability. Associated lumbar compressive forces may be inappropriate for some people with compressive intolerant lumbar spine pathology[38].

When the inclination of the pelvis was voluntary increased, there was an increase in the erector spinae muscle activity and when decreased there was a decrease in the muscle activity[39] . The increased activity of the erector spinae occurs in the longissimus and iliocostalis of the thoracic part and in the multifidus. The increased activity of these muscles can be reduced through latissimus dorsi exercise, as the latissimus dorsi complement the erector spinae[21].commercial exercises (torso track and AB slide) generate tension in the latissimus dorsi in addition to the internal oblique which all tense the thoracolumbar fascia and enhance trunk stabilization but maintain the spine and pelvis in neutral or extended position[33] which are not suitable to this subgroup in the current study. Latissimus dorai muscle EMG was high using some commercial device (power wheel pike , power wheel knee up and hanging knee up with strap) ,however they show highest lumbar paraspinal EMG and that rectus femoris was highest for the power wheel Knee up. In addition that the subjects

participated in that study were all relatively young, active people. Older, less active or people with trunk pathology may not be able to perform the more difficult exercises used in that study[18]. These commercial exercises did not concentrate on the transverse fiber of the latissimus dorsi compared with that in the current study, hence in this study through strengthening of the latissimus dorsi specially the part attached transversely to the thoracolumbar fascia, active tension is developed in the fascia which ensheaths the back muscles, preventing their dorsal displacement, hence its action can be done with less effort.

One of the most common clinical challenges is to stretch the hip joint capsule and anterior thigh musculature without creating an extension force to the lumbar spine [1].The method used for stretching hip flexor in this study has several advantages. The starting position for stretch posteriorly rotates the pelvis, this implies a decreased risk of hyperextension, the more posterior tilt pelvis in this position means greater extension of the hip[40].The pelvis is stabilized by a designed belt to reduce the magnitude of electrical activity in the sacrospinalis ,following the study of **Fisher and Houtz[30]** the designed stretch tool allowed low load prolonged stretch technique .Permanent lengthening is most favored by low force longer duration stretch[41].

Abdominal drawing in maneuver during prone hip extension exercises is recommended as an effective method for preventing excessive anterior pelvic tilt and increase the contribution of the hip extensors while reducing the activation of the lumbar erector spinae .While the drawing in maneuver decrease anterior tilt from 10 to 3degrees, the starting position in the current study was the starting position of stretch, which may eliminate any degree of anterior pelvic. In addition that Drawing in maneuver may preclude hip extension in the prone position hip flexion contracture and significant weakness of the gluteus maximus[42].So in the current study, in the first month of treatment initial attention is focused on gluteal setting exercises which elicited a large degree of activity regardless of the position and low load stretch of hip flexor preceded isotonic hip extension .In the second month of treatment hip extension is performed while the hip is abducted and externally rotated. This exercise exhibit the strongest contraction of the gluteus maximus[30] .In the current study hip extension is accompanied with knee extension, So almost all rectus femoris activity disappears as well as no activity in the hamstring[43]. Hip extension in combination with lateral rotation might be effective choices to optimize gluteus maximus function[44].

Specific exercise treatment approach was more effective than general conservative treatment [45,46] .The current program found a significant reduction in pelvic tilt and lumbar curve in contrast with **Lindgren *et al.*** [47]. Who did not find any radiographic improvement in 9 subjects with segmental dysfunction, following a treatment regimen focusing on strengthen of the abdominals and multifidi. The use of subgrouping classification methods for the physical therapist management of subjects with LBP may result in better outcomes than physical therapist management that is not classification based. Using specific inclusion criteria to identify more homogenous subgroups of subjects and attempting to match treatment to the subgroup has the potential to enhance treatment effects [48,18,49]. The findings of **Browder *et al.*[50]** support the hypothesis of improved outcomes when interventions are matched to more specific subgroups of patients, hence this program focused on subgroup of adults complaining of chronic low back pain associated with increased lumbar curve.

Conclusion

The current study introduced newly developed exercise program succeed to decrease pelvic tilt and consequently accentuated lumbar lordosis and support the relation between low back pain and accentuated lumbar lordosis.

The design as well as effect of the designed stretch tool on the hip rang of motion will be discussed in a separate article.

Key points

- The study introduced newly developed exercise program to decrease pain associated with the accentuated lumbar curve.
- Find a correlation between the change of pelvic tilt and lumbar lordosis.
- Studies comparing X ray measurements pre and post treatment to detect the role of accentuated lumbar curve in LBP should be done, rather than studies comparing patients with low back pain with normal subjects. There is wide a rang of normal lordosis which mask any difference between both group studies.
- The study only limited to those patients who had Cobb angle measurements more than 50,therefore all patients had lumbar lordosis below this angle were excluded from the study.

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Forward Head Correction Exercises For Management Of Myogenic Tempromandibular Joint Dysfunction

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Abstract: Objectives: To investigate the effect of independent forward head postural exercise on myogenic tempromandibular joint dysfunction. Methods :The study included fifteen females , diagnosed as myogenic TMJ dysfunction with limited mouth opening aged between 20-40 years(27.1± 4.6 years).TMJ pain was Persistent for at least six months(12.3±5.3) and Patients were observed to have a forward head posture .The measurements included vertical mouth opening measured in millimeters by Standard ruler, pain intensity using visual analogue scale and craniocervical posture on lateral cephalometic .Each patient received exercise program consisting of 1- strengthening exercise of deep cervical flexors and scapular retractors 2- stretching exercise of the suboccipital muscles and pectoralis muscles. Results: The result showed significant decrease in crainocervical angle and lower cervical curvature. Also there was a significant increase in vertical active mouth opening (P=0.000) and decreased pain level of masticatory system (P=0.000). The correlation between mouth opening and the Craniocervical angle was found to be significant .No significant correlation was found between upper and lower cervical curvature. Independent forward head correction exercise program was found to be effective in improving myogenic TMJD and support the relation between forward head posture and TMJD.
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Keywords: Myogenic; tempromandibulr joint; dysfunction; myofascial pain; forward head posture; posture exercise.

1. Introduction:

Tempromandibular disorders can be subdivided into muscular and articular categories .Myogenic disorders include myalgia (myofascial pain, fibromyalgia), myospasm, splinting, and fibrosis/contracture [1] Tenderness to palpation of muscle, limitation in mandibular range of motion, perceived alteration in the dental occlusion, and changes in mood are conceptualized as consequences of myogenic pain [2]. It has been reported that approximately 50% of all TMDs are masticatory myalgias or painful masticatory muscle disorders[3]. Within Myogenic Disorders myofacial pain (MFP) and myofacial pain dysfunction syndrome (MPD) are encountered frequently. Patients suffering from MFP will have tenderness to palpation of two or more muscle sites. Myalgias involving the muscles of mastication predominate MFP escalates to (MPD) when there is concomitant limitation of jaw opening[4].Many researchers have examined the role of estrogen in the etiology of masticatory myalgias [5], the fact that the condition is more sever in women than men,and that it occurs mor frequently in women of reproductive age bears further investigation[1].

Forward head position is characterized by an extension of the head together with the upper cervical spine (C1 to C2) accompanied by a flexion of the lower cervical spine (C4 to C7) This posture is associated with weakness in deep cervical short flexor muscles (capital flexors), and mid thoracic scapular retractor (i.e., rhomboids, middle and lower fibers of trapezius) and shortening of the opposing cervical extensor and Pectoralis muscles [6]. When the head is positioned forward the upper trapezius muscles activity is significantly higher than it is when in the normal alignment, the more the patient is to have pain from overusing the muscles[7]. Forward head posture mostly occurs by the weakness of the anterior cervical neck flexor muscles which result in tightness of the sternocledomastoid [8].Forward head posture and flexion of the trunk form the main components of slumped sitting. When sitting in the slump sitting posture, the muscle activities of the serratus anterior, middle trapezius muscle and external abdominal oblique were significantly lower and that of the masseter, upper trapezius, L3 paraspinal muscles were significantly higher[9].

There is a significant relation ship between forward head posture and tempromandibular disorders [10]. The cervical muscle activity influnces the masticatory muscle activity[11]. Forward head posture produces a greater muscle activity in the temporal and masseter muscles [12] .The muscle activity resulting from craniocervical extension of the head produces an elevation and retrusion force that act on the mandible which results in decrease in free

way space of TMJ [13]. Some studies have investigated the relationship between the masticatory muscles and head posture using electromyographic (EMG) analysis [14].There is higher resting activities of sternocledomastoid and trapezius muscles of myogenous TMD patient[15].The functional link between the masticatory and cervical muscle is probably through a co-activation mechanism [16,17]. Most cervical muscle myofascial trigger point (MTrPs) have referred pain pattern into the head and face region [18] Carlson[19]demonstrated the clinical significance of referred pain by eliminating pain of the masseter muscle by treating MTrPs in the trapezius muscle.

Two studies examined the effect of postural training in combination with other therapies on myogenic TMDs [20,21]. As independent effects of postural training on myogenic TMDs are unknown[22]. So, the purpose of this study was to investigate the forward head posture correction in the management of myogenic temporomandibular joint dysfunction and in changing of craniocervical posture.

Patients and Methods

The study was conducted in the Department of Oral and Maxillofacial Surgery, Faculty of Oral and Dental Medicine, Cairo University. Fifteen subjects were selected consequently and agreed to participate in this study, they were all females, they met all of the following criteria 1) Patients diagnosed as myogenic TMD with limited mouth opening. 2) Age range between 20-40 years (27.1± 4.6). 3) Persistent temporomandibular joint pain for at least six months. 4) Patients were observed to have a forward head posture. Subjects exhibiting the following conditions were excluded from the study.
1) Temporomandibular joint dysfunction includes disc displacement, arthritis or arthralgia. 2) Recent macro trauma history in the head or cervical area. 3) History of previous posture correction treatment. 4) Recent surgery in head or neck. 5) Patients currently receiving medication or other treatment. 6) Patients with upper respiratory or major psychological problems.

Measurements

Patients were evaluated for pain intensity, range of motion (ROM) of active mouth opening, and craniocervical posture before and after the treatment. Visual analogue scale was used to measure pain. The scale is straight undivided line 10 cm in length, the ends of which is marked by statement indicating the extreme limits of pain sensation to be measured, that is "No pain" at the far left end, and "extreme Pain" at far right end (Subjects was asked to record the intensity of their pain complain on a visual analogue scale (VAS). Pain score will be obtained by measuring the distance in millimeters from the far left end of VAS). vertical mouth opening was measured in millimeters by Standard ruler, each patient was asked to open her mouth as wide as possible without feeling any strain and without causing pain and discomfort .The intercisal distance was measured by placing one end of the ruler against the incisal edge of one of the upper central incisors, and the other end against the incisal end of the opposing lower incisor, then the opening range will be recorded in millimeters.

X-rays were taken by the operator with the patient's mid- sagittal plane parallel to the X- ray film plane, feet together in an orthostatic posture. The patient was instructed to look towards the horizon [23]. It is important that the operator does not grab or push the head or neck with the hands since this will change the cranio-cervical angle. Four lines were traced on lateral cephalometric. The relationship between the head and the cervical spine(craniocervical angle) is expressed as the angle between the line from the posterior nasal spine to lower surface point of the occipital bone (McGregor plane) and line tangent to the odontoid apophysis posterior surface. The lower cervical curvature is measured and expressed by the angle between the line extended from the posterior margin of the third cervical vertebra and up from the posterior margin of the six cervical vertebral body as shown in fig 1 a as the seventh cervical vertebra is not well clear in some x rays), hence the lower cervical curvature is measured from C3- C6 instead C4- C7.The measurements were taken on x rays using a protractor.

Exercise therapy

A six week program was conducted. Each patient received three sessions per week day after day. The program included two categories of exercises. In each session the exercises were practiced and any error observed was corrected. The program was based on a program by **Harman** [6] .These exercises were:

Strengthening exercises

Strengthening exercise was preceded by kinesthetic training .The goal of kinesthetic training was to develop proprioceptive awareness of posture, positioning and safe movement. . Reinforcement technique was used in kinesthetic training such as verbal reinforcement and tactile reinforcement [24].

Strengthening of deep cervical flexor muscles

Each patient was instructed to sit with her arms relaxed at the side. The area above the lip and under the nose was lightly touched and the patient was asked to tuck her head down and in. The correct movement of tucking the chin in and straightening the spine was verbally reinforced. From sitting position the patient then was asked to tuck her chin so that her ears were in line with the tip of her shoulders. The exercise was performed for three sets of 12 repetitions with holding of six seconds.

Strengthening of the scapular retractor muscles

The patient sat on a chair without back support, tactile and proprioceptive training is preceded. The movement of the inferior angle of the scapula was gently resisted and the patient was asked to pinch them together "retraction". The patient was asked to imagine "holding a quarter between both the shoulder blades". Each patient was instructed not to extend the shoulders or elevate the scapulae. The patient then stood with her hands grasped together behind the lower back (this activity cause scapular adduction). She was instructed to adduct scapula and hold the adducted position with both arms lowered downwards for six seconds. This exercise was performed for three sets with 12 repetitions with holding 6 seconds.

Stretching exercises

Stretching of suboccipital muscles.

The exercise was performed from sitting position. The spinous process of the second cervical vertebra was identified, and stabilized by the therapist's thumb. The patient was asked to slowly nod, doing just a tipping motion of the head on the upper spine. The exercise was done three times with holding 30second each time.

Stretching of the Pectoralis major muscle

This exercise was done from sitting with hands behind the head, shoulders abducted and externally rotated 90 degree. Passive stretch was applied by the therapist at the end of range. This exercise was done three times with holding 30 seconds.

Statistical analysis

Paired T test and correlation coefficient were used to judge statistical significant difference. The level of significance used was $p<0.05$. All data were analyzed using SPSS program version 12.0

1- Paired T test for craniocervical angles, mouth opening and visual analogue scale of pain.

2- Correlation between the change of upper and lower cervical angles.

3- Correlation between the change of craniocervical angles, mouth opening, intensity of pain.

Results

A total of 15 female patients participated in this study. The mean value of "age" was 27.1 ±4.6 years. They received an exercise program for correcting forward head posture. The mean value of duration of temporomandibular dysfunction in the sample selected was (12.3± 5.3).

There was a significant reduction in craniocervical angle, lower curvature of cervical spine (c3-c6), and pain. There was also a significant increase in mouth opening in Table 1. It was noticed that all cases show reduction in cervical curvature post treatment except one case show increase in cervical curvature as shown in Figs. 1a,b. There was a significant correlation between the change in craniocervical angle and change in range of motion equal to -.519, P value = .047.There was a correlation between range of motion and lower cervical curvature but not significant . A significant correlation was found between the change of VAS of pain and only the lower cervical curvature equal to -.596. The change of VAS of pain is well correlated with change in range of motion, the correlation equal to-.704 Table 2. No significant correlation was found between craniocervical angle and lower cervical curvature before treatment. The correlation improved post treatment but still not significant Table 3.

Table 1: Comparison between pre and pos treatment variables(postural angles, mouth opening and VAS of pain.

	Pre-Treatment	Post-treatment	Mean difference	T value	Significance
Craniocervical angle	99± 8.	87.9±7.3	11. ±6.5	6.56	.000
Lower cervical curvature	18±10.3	7.4±10.7	10.6±10.4	3.9	.001
Mouth opening	24.9±2.2	41.2±2.7	-16.2±3.2	-19.717	.000
VAS* of pain	7.4± .75	2.3±1.5	5.14±1.5	13.251	.000

*Visual analogue scale.

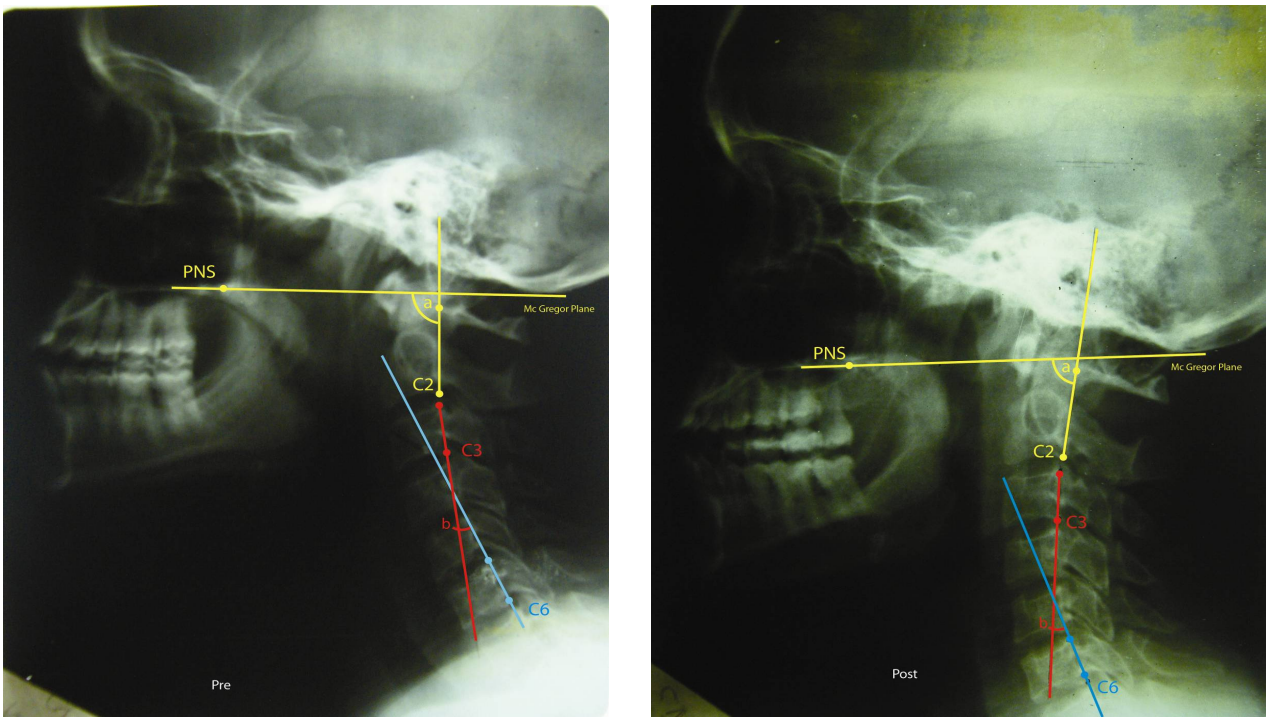
Table 2: Correlation between difference of pre and post treatment for the postural angles, range of motion and VAS of pain

Variables	Correlation	Significance(2tailed)
Craniocervical angle & Range of motion	-.519*	.047
Cervical curvature & Range of motion	.479	.071
VAS Pain & Range of motion	-.704**	.003
VAS of pain & Crainocervical angle	.401	.139
Lower Cervical curvature & VAS of pain	-.596*	.019

* Correlation is significant at the 0.05 level (2-tailed) * * Correlation is significant at the 0.01 level (2-tailed)

Table 3 : Correlation between upper and lower curvature pre and post treatment

Craniocervical angle & lower cervical	Correlation	Significance
P re-treatment	**.127**	**.652**
Post –treatment	**-.442**	**.099**



(a) Pretreatment **(b) Post treatment**

Fig .1. lateral X-ray measurement for craniocervical angle (a) and cervical curvature angle (b).

*** PNS: Posterior nasal line**

4. Discussion

The current study investigated the independent effect of forward head postural correction in the management of myogenic temporomandibular joint disorder. The program is not combined with other techniques as previously don by Komiyamama *et al.*[20].The study gives details about posture correction on mechanical basis which is not found in other study [21]. The studies done by Komiyamama *et al.*[20] and Write [21] were considered weak studies according to JADA score because the treatment protocols were not described in adequate details to allow replication of the intervention used in other researches [25]. On the other hand, in this study the patients complaining from myogenic TMD received a program of six week postural correction. This program was based on a mechanical aspect that forward head posture is associated with weakness of deep cervical flexors (craniocervical flexor) and scapular retractors and shorting of suboccipital muscles, and Pectoralis muscles [6].

This study showed a significant decrease in two angles, the craniocervical posture angle and the angle of the lower cervical curvature. The exercise program in this study included strengthening of the deep flexors of the neck along with stretching of suboccipital muscles. Since the deep cervical flexors have a major postural role in supporting and straightening of the cervical spine [26] and normal balance of the head and neck unit requires balance of the anterior and posterior muscles [27]. Our finding disagrees with Luen [28] who suggest that posture should not be interpreted as an etiological factor predisposing TMJD.

Results of the current study agree with Harman *et al.*[6] who reported improvement in head posture by using program of posture correction similar to the program of this study. The results of this study disagree with Wright *et al.*[21] who did not find any improvement in head posture in patients with myogenic temporomandibular disorder receiving posture correction program. This can be explained by the fact that his study used a program of treatment that included neck retraction training and stretching of the anterior chest muscles in addition to strengthening of shoulder retractors without including stretching of the suboccipital muscles and the duration of the treatment was four weeks (performed as a home regime only and the therapist saw subjects once only during the treatment to correct errors). Also the subject's age range for that study was too wide (ranged from 18 to 56 years) .Other studies concerning posture correction for the treatment of myogenic TMJD didn't measure head posture[20, 29].

There is a significant correlation between change in range of motion and changes in craniocervical angle which may support the relation between forward head posture and TMJD, but the change in VAS was significantly correlated with the lower cervical curvature. Improvement of pain and range of motion of mouth opening may also be due to the fact that exercise causes relaxation of the neck muscles thereby relaxing masticatory muscles and so decreasing pain and increasing range of motion.

A weak statistically significant negative correlation in asymptomatic volunteers females was observed between the measured angles of the upper and lower cervical spine [30], this may be due to the wide variation in the degree of cervical curvature from kyphosis to lordosis. Non lordosis or angular kyphosis have been reported to be often observed in the normal population [31]. In the current study the correlation between the upper and lower cervical spine improved post treatment but still not significant. A further study needed to investigate the relation between upper and lower cervical in myogenic TMJ disorders.

Proper alignment of the upper back is essential for proper alignment of the head and neck .Round upper back (thoracic kyphosis) which affect cervical posture is associated with weakness of thoracic spine extensor muscles [32]. Magee [33] reported that forward head posture and flexed upper back are characterized by weakness of the lower cervical and thoracic erector spinae. Falla [26], found that forward drift of the head was associated with an increase in the thoracic flexion curve in patients with neck pain, our program did not include strengthening of thoracic extensor muscles to improve round upper back posture if any. McDonnell [34] suggests that impairments, not only in the cervical region, but also in the scapulothoracic and lumbar regions, may be important to consider in the treatment of cervicogenic headache.

Conclusion

Correction exercise program of forward head posture is effective in improving head posture and range of motion of active mouth opening and reducing pain of the masticatory system. The data of this study support the relation of forward head posture and myogenic TMJD. It is recommended that impairments, not only in the cervical region, but also in the scapulothoracic and lumbar regions should be corrected thus indirectly decrease the prolonged loading into extension in the cervical region .A further investigation of the effect of postural correction on the relation between upper and lower cervical spine is recommended.

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Inhibitory Effect of Some Cationic Gemini Surfactants for Carbon Steel in Sea Water

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Abstract Corrosion inhibition of carbon steel alloy in sea water by different synthesized gemini surfactants {12-2-12, 14-2-14 and 16-2-16} have been investigated using weight loss, potentiodynamic polarization and surface tension measurements. The data obtained from all the used methods are in good agreement with each other and ensure the excellent inhibition efficiency of the tested surfactants for carbon steel in sea water. The inhibition efficiency increases with increasing the concentration of the studied inhibitors. Also, the adsorption ability of the surfactant molecules on carbon steel surface increases with the increase in the hydrocarbon chain length of the surfactant molecule from 12 to 16 and through 14 C atoms; meanwhile, the isotherm of 16-2-16 declares the formation of multilayer onto the used metal surface. The inhibitive action of the studied surfactants follows the order: 16-2-16 > 14-2-14> 12-2-12. The morphological changes of carbon steel surface were studied by Scanning Electron Microscope [SEM]] and the study shows well inhibited surface on adding the cationic Gemini surfactants to the immersion solution .

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Keywords: Carbon steel, sea water, gemini surfactant, weight loss, potentiodynamic, adsorption onto Solid/liquid interface.

1. Introduction:

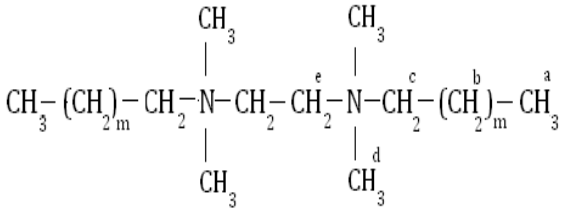
Low carbon steel is being used extensively under different conditions in industries because of its low cost and excellent mechanical properties. However, some corrosion problems take place due to the use of natural sea water in cooling systems, storage reservoirs, and water transport pipelines for injection systems [1]. In order to reduce the corrosion of metal, several techniques have been applied, where among that utilization of organic compounds and more specifically cationic surfactants are gaining high space as corrosion inhibitors. Surfactants are very beneficial reagents and their presence at very low quantity in any medium providing desirable properties to processes in all industries such as, petrochemical, food, paint and coating industry [2]. As a new generation of surfactants, gemini surfactants have attracted great interest in recent years. This kind of surfactant contains two hydrophilic groups and two hydrophobic groups in the molecule, separated by a rigid or flexible spacer, rather than one hydrophilic group and one hydrophobic group for conventional surfactants [3]. Gemini surfactants are about 3 orders of magnitude more efficient at reducing surface tension and more than 2 orders of magnitude more efficient at forming micelles than conventional surfactants [4]. Many gemini surfactants have been synthesized, and a considerable number of investigations have been reported on their unusual physicochemical properties, including their high surface activity, unusual changes of viscosity, unusual micelle structure, and aberrant aggregation behaviors [5-13]. In general, cationic surfactants and particularly gemini surfactant possessing effective inhibitory effect, they accumulate in special order at the interfaces and modify the interfaces and thus, control, reduce, or prevent reactions between a substrate and its surroundings when added to the medium in small quantities [14].

In the present study, a series of gemini cationic surfactants with different chain lengths n-2-n (n = 12, 14, and 16 C atoms) are synthesized, purified, and characterized. Surface tension, weight loss, potentiodynamic polarization measurements, and scanning electron microscopy (SEM) were performed to examine the structural inhibitive effect of these tested inhibitors on the corrosion process of carbon steel in natural sea water. The adsorption mechanism of the gemini surfactants onto the carbon steel surface is discussed in order to shed light on the inhibition mechanism of the tested inhibitors.

2. Material and Methods

2.1. Synthesis

A series of cationic gemini surfactants N-alkanediyl 1, 2-ethane bis (dimethyl ammonium bromide) namely: 12-2-12, 14-2-14, and 16-2-16 were synthesized, then purified, and characterized. The structure is shown in the following scheme



Molecular structure of the studied Gemini surfactant

where m= 10, 12, or 14

The tested surfactants were prepared from N, N, N', N'-tetramethylethylene diamine and the corresponding alkyl bromides of n = 12, 14, and 16 in absolute ethanol under reflux for 24 hours. Ethanol is removed via rotary evaporator where a waxy product was obtained. The resulting product was extracted, using diethyl ether and recrystallized from acetone/ethyl acetate mixture [15].The chemical structure of the prepared surfactants was confirmed by the following techniques:

a) Elemental analysis

Elemental analyses were performed for the prepared surfactants via Elemental Analyzer Perkin Elmer 240 C, and the obtained results are given in Table (1).

b) ^{1}H-NMR

^{1}H-NMR for the prepared surfactants was measured using Jeol-EX-270 MHz NMR Spectrophotometer. The following bands were shown (MHz, δ, $CDCl_3$/TMS): (a) t, 0 .87 ppm; (b) m, 1.23 ppm; (c) m, 1.79 ppm; (d) s, 3.14 ppm; (e) m, 3.55 ppm.

c) Mass spectra

The prepared surfactants were analyzed via mass spectra using GC Mass-Qt 1000 EX Schimatz Spectrophotometer, Japan E.I. 70 EV. The giving data were:

12-2-12: (m/z = 613.8; $[M+ 1-^{81}Br]^+$ = 533.4; $[M-2Br/2]^+$ = 226
14-2-14: (m/z = 669.8; $[M+ 1-^{81}Br]^+$ = 593.5; $[M-2Br/2]^+$ = 254.1
16-2-16: (m/z = 725.8; $[M+ 1-^{81}Br]^+$ = 675.5; $[M-2Br/2]^+$ = 332.1

2.2. Solution

The sea water used in this study was collected from Mediterranean Sea (Alexandria-.Egypt). Its constituents were defined by Atomic absorption spectroscopy (AAS) technique and the obtained results are given in Table (2).

2.3. Surface Tension measurements

The surface tension at air/liquid interface of the tested surfactants was measured in sea water solution of different concentrations (2.0 x 10^{-5}- 6.0 x 10^{-3} mol dm^{-3}) by Krüss Tensiometer K 6 at 25°C using the Platinum ring method.

2.4. Adsorption Measurements

The adsorption isotherms of the tested surfactants onto carbon steel surface were obtained by the immersion method (at 25 °C). Coupons, of the used metal, were equilibrated for 144 hours in 25 ml of surfactant solutions of known concentrations ranged from 2.0 x 10^{-5} mol dm^{-3} to 6.0 x 10^{-3} mol dm^{-3}. The amount adsorbed was calculated from the changes in concentration of the solutions using the curves given in Fig. 1 as a calibration curve, according to the following equation,

$$\Gamma = \frac{(C_o - C_{eq})V}{m} \qquad (1)$$

where Γ is the amount adsorbed in m mol g^{-1}, $C_{\square}$ is the initial surface concentration in m mol dm^{-3}; C_{eq} is the surfactant concentration at equilibrium in m mol dm^{-3}; V is the volume of the liquid phase in liter; and m is the mass of adsorbent in gram.

2.5. Gravimetric Measurements

Rectangular specimens of low carbon steel (2.5cm × 2.0 cm ×0.5 cm) were abraded with 180, 400, 600, and 1200 grades of emery paper they were degreased with acetone and rinsed with distilled water two times. Then, they were immersed in 0.5 M HCl solution for 10 sec, rinsed with distilled water two times and finally dried. The specimens were accurately weighted and then immersed in 25cm^3 of sea water with and without different concentrations of the studied surfactants. After 144 hrs, the steel sheets were taken out, rinsed thoroughly with distilled water, dried, and reweighted accurately. The weight loss of the carbon steel sheets was obtained. .The chemical composition of the used carbon steel sheets is listed in Table (3).

2.6. Electrochemical Measurements

Electrochemical experiments were carried out using Potentiostat Galvanostat **(**EG & G model 273**).** Standard ASTM glass electrochemical cell was used. Platinum electrode was used as auxiliary electrode .All potentials were measured against saturated calomel electrode (SCE) as a reference electrode. The working electrode has a form of square with a surface area of 2.25 cm^2. All experiments were conducted at room temperature. Potentiodynamic

polarization curves were obtained by changing the electrode potential automatically from -1000 to +1000 mV with a scan rate 10 mvs^{-1}.

2.7. Scanning Electron Microscopy (SEM)

The surface examination of carbon steel samples in sea water, without and with the optimum concentration of tested inhibitors was performed using scanning electron microscope (Jeol 5400). The energy of the acceleration beam employed was 30 KV.

3. Results and Discussion

3.1. Surface properties

The surface tension values (γ, mNm^{-1}) for the cationic gemini surfactants under investigation were plotted against the logarithm of the surfactant concentration (Log C, mol dm^{-3}) as shown in Fig. 1 (a, b and c) for 12-2-12, 14-2-14 and 16-2-16, respectively. The critical micelle concentrations (CMCs) of the tested surfactants were determined from the point of intercept of the two linear portions obtained by the γ-Log C plots.

It is clear that, for all the tested cationic gemini surfactants, the surface tension decreases with increasing the concentration until CMC is reached, above which the surface tension is not affected by a further increase in the surfactant concentration.

The obtained CMC values for the studied surfactants are reported in Table (4). The surface excess concentration $\mathbf{\Gamma_{max}}$ (in Mol cm^{-2}) and the surface area per molecule A_{min} (in nm^2) for the investigated surfactants were calculated according to the following equations [16]

$$\Gamma_{max} = -1/nRT\ (\partial \gamma / \partial \ln C)_{T,P} \qquad (2)$$

where n=3 for ionic gemini surfactants

$$A_{min} = 10^{-14}/N_A\ \Gamma_{max} \qquad (3)$$

where N_A is the Avogadro's number.The obtained data is reported in Table (4).

It is observed that the surface value of A_{min} increases with increasing the hydrophobic chain length of the tested surfactants. This is because A_{min} depends primarily on the adsorption interface which is, in turn, affected by the total number of carbon atoms in the chain and hence on the hydrophobicity of the surfactant [17].

3.2. Adsorption of the tested surfactants onto carbon mild steel surface

The efficiency of an inhibitor in inhibiting the metal corrosion depends on the extent to which this inhibitor adsorbs onto that metal surface and the configuration in which they adsorb. So, measurements of adsorption isotherms can lead to a better understanding of the corrosion inhibition behavior of the tested surfactants.The amount of surfactant adsorbed onto the tested carbon mild steel surface, Γ in m mol g^{-1}, has been determined as a function of surfactant concentration and plotted against the surfactant equilibrium concentration C_{eq} (in m mol dm^{-3}) at 25ºC as shown in Fig 2 (a, b, and c) for the tested gemini surfactants 12-2-12, 14-2-14 and 16-2-16, respectively.

The adsorption isotherms of all the investigated cationic gemini surfactants exhibit segmoidal S-shapes as shown in Fig (2). The formation of multilayer onto the adsorbent surface represents a strong interaction between the adsorbed molecules themselves whereby cooperative adsorption occurs in the hydrocarbon chains [18].

Close looking at the isotherm of 12-2-12 illustrated in Fig 2 a displays the missing of the first horizontal region of the S-shape isotherm, this is attributed to the positive charge on the metal surface, which drives electrostatic repulsion that hinder adsorption of cationic surfactant [19]. This electrostatic repulsion is overcome in case of 14-2-14 & 16-2-16 due to the increase in the length of their hydrocarbon chains as illustrated in Fig 2 b & c where region I is clearly shown. This may be related to the increase in the hydrophobic properties of the surfactant molecule, which, in turn, increases its ability of forcing the surfactant molecules to adsorb onto the metal surface in order to improve the thermodynamic balance of the system. At the beginning of region I, in case of 14-2-14 & 16-2-16, and at low concentration below their CMCs, the adsorption is taken place at the cathodic charged surface sites result from the anions present in the used sea water (Table 2). The adsorption in this step is taken place by an electrostatic ion-exchange mechanism.

In spite of the presence of electrostatic repulsion, adsorption of the cationic gemini surfactant 12-2-12 onto the tested metal surface is taken place in region II. At the beginning of this region and at low concentration below CMC of the tested surfactant, the adsorption is taken place by an electrostatic ion-exchange mechanism. This result declares that the electrolytes present in the used sea water help the adsorption of this surfactant onto the investigated metal surface, which possesses +ve charged sites. This fact is in concomitance with that recorded by Rosen [20]. Also, Asefi et al. [21] deduced that the inhibition efficiency of gemini surfactants increases with the presence of halide salt. The head groups of the adsorbed molecules are firstly

oriented toward the metal surface while their hydrophobic tails lay horizontally or titled forming a monolayer onto the substrate. By increasing the surfactant concentration, surfactant molecules adsorb onto the metal surface by cooperative hydrophobic interactions of the oncoming surfactants ions with the already adsorbed molecules and with themselves. This aggregation is known as hemimicelle formation with the adsorbed molecules oriented perpendicularly toward the tested substrate where their head groups faced the bulk solution. At the end of region II, the metal surface has acquired a charge of the same sign as the surfactant ion. The adsorption in region III must overcome the electrostatic repulsion acting between the oncoming ions and the similarly charged solid, therefore, the slope of the isotherm in this region is reduced and the adsorption in case of 12-2-12 and 14-2-14 is leveled off when the surface is covered with the surfactant molecules (Figs. 2 a & b). This behavior is in line with that previously published [22].

The increase in the hydrophobic chain length from 14 C to 16 C leads to the increase in the slope of the isotherm as represented by region IV for the tested surfactant 16-2-16 (Fig. 2 c). This event may be attributed to the formation of multilayer or a sort of aggregation of the adsorbed molecules onto the used metal surface.

3.3. Weight loss tests

The weight loss tests are carried out in sea water in the presence of different concentrations of the tested surfactants (12-2-12, 14-2-14, and 16-2-16) at 25ºC. For all the investigated surfactants, a decrease in the weight loss of carbon steel in the presence of these additives is observed as general trend even at a low concentration compared with the surfactant free solution.

The corrosion rate **(*K*)** was calculated using the following equation [23]:

$$K = W / St \quad (4)$$

where W is the weight loss of the carbon steel sheets, S is the surface area of the specimen, and t is the immersion time. Also, the inhibition efficiency (η_w%) and surface coverage (□) were calculated according to the following equations [24] :

$$\eta_w\% = (W_{corr.} - W_{corr.(inh)} / W_{corr} \times 100 \quad (5)$$

$$\square = (W_{corr.} - W_{corr.(inh)} / W_{corr.} \quad (6)$$

where, $W_{corr.}$ and $W_{corr.(inh)}$ are the weight loss of carbon steel in absence and in presence of the inhibitors, respectively. The obtained weight loss data of the carbon steel in sea water and in presence of various concentrations of inhibitors are recorded in Table (5). The results show that all the tested surfactants act as good corrosion inhibitors for carbon steel in sea water but with different extents. Also, the obtained data (Table 5) show an increase in the corrosion inhibition efficiency with the increase in the surfactant concentration.

Generally, it is agreed that the primary action in the inhibition process by surfactants is the adsorption of the surfactant molecules via their functional group onto the metal surface. As indicated from the data reported in Table (5), the higher the degree of the surface coverage the lower is the corrosion rate of carbon steel and hence the higher the inhibitor efficiency. This behavior is confirmed by the surface adsorption study for the used surfactants as shown in Figs. (2 a, b, and c). The trend presented by the adsorption isotherms given in this figure is in line with behavior shown in Fig.(3).

It is clearly evident from Table (5) and Fig.(3) that the cationic gemini surfactant 16-2-16 exhibits the highest extent of surface coverage among all the tested inhibitors, corresponding to the best inhibition effect. The value of (□) in the presence of this surfactant approaches unity, indicating almost a full coverage of the surface with the adsorbed surfactant molecules. These appear to act as a good physical barrier shielding the corroding surface from the corrosive environment and thus bringing down the corrosion rate of carbon steel very significantly. As previously mentioned, this surfactant form a multilayer or sort of aggregation onto the tested metal surface (Fig. 2 c), which provides a good hydrophobic barrier against the corroded bulk solution.

On the basis of the view that corrosion inhibition by surfactants depends on the ability of these compounds to adsorb onto the corroding surface which is directly related to its capacity to aggregate to form clusters (micelles). The critical micelle concentration (CMC) can be considered as a key factor in determining the effectiveness of surfactants as corrosion inhibitors. It is well known that for a surfactant to be an excellent corrosion inhibitor it should exhibit a low CMC value, i.e., the inhibition effectiveness is inversely proportional to the CMC value [25]. This fact is clearly achieved fulfilled in this research where 16-2-16 exhibits the lowest CMC value among all the tested surfactants (Table 4) and possesses the highest efficiency in inhibit the tested metal surface against corrosion in sea water (Table 5).

The corrosion inhibition efficiencies for all tested surfactants, as shown in Fig (3), increase with increasing the hydrocarbon chain length following the order 16-2-16> 14-2-14 >12-2-12. This result

indicates that the adsorption ability of the investigated surfactants increases with the increase in the chain length of the surfactant molecule and hence improves its inhibitive action.

3.4. Potentiodynamic polarization measurements

Potentiodynamic polarization tests were performed on the carbon steel under investigation in the used sea water with and without the presence of different concentrations of tested surfactants. Fig.(4) illustrates an example of the anodic and cathodic polarisation curves measured in sea water with optimum concentration of the tested surfactant 16-2-16 since it showed the best performance. Also, the polarization curves with and without the addition of optimum concentration of 12-2-12 and 14-2-14 inhibitors are shown in Fig.(5). The typical Tafel polarization curves obtained for different concentrations of the investigated surfactants and blank show a change in shape of the cathodic and anodic curves.

Various corrosion parameters such as corrosion potential (E_{corr}), corrosion current density (i_{corr}), and percentage of inhibition efficiency (η_{pot} %) are given in Table (6). The corrosion current obtained for the tested carbon steel coupons in all surfactants solutions under investigation are lower than inhibitor free solution, i.e. blank. These lower corrosion current values for inhibitor solutions imply that the rate of electrochemical reactions are reduced due to the formation of a barrier layer over the carbon steel surface by the inhibitor molecules [26]. An increase in the inhibition efficiencies with the increase in the inhibitor concentration is observed in Table (6) for all the tested inhibitors where the highest value of inhibition efficiency (η_{pot}%) is observed at the surfactant concentration 6 m mol dm^{-3} for all the tested surfactants. This result is in parallel with that obtained from the surface study where the adsorption isotherms given in Figs.(2 a, b, and c) for these surfactants show an increase in the adsorption with the increase in the surfactant concentration.

Weight loss and polarization results show that the tested surfactant 16-2-16 possesses the best inhibition action in comparison with all the tested surfactants and appears to create a good hydrophobic physical barrier to the aggressive ions, this barrier accounts for its high inhibition efficiency. This result is confirmed by the surface study where the adsorption isotherm illustrated in Fig 2 c for this surfactant demonstrates the formation of multilayer onto the used metal surface as previously discussed.

The data reported in Table (6) also reveals that E_{corr} values of inhibited and uninhibited systems do not vary significantly indicating that both anodic and cathodic reaction are affected by the addition of the studied surfactants. This behavior leads us to suggesting that the studied cationic gemini surfactants are mixed type (anodic/cathodic) inhibitor [27, 28].

The good inhibitive action of the tested cationic gemini surfactants can be related to the fact that, quaternary ammonium salts have been used extensively as inhibitors against the corrosion of iron and steel, and this kind of organic molecules can be adsorbed on the metal surface because it can form a bond between the polar head groups and the metal surface thereby, reducing the corrosion attack on the metal surface [29].

It is known that the gemini surfactant molecule consists of two hydrophilic groups and two hydrophobic groups that results in a complicated adsorption of this kind of surfactants onto metal surfaces. As previously published [30], three different scenarios can exist for the monolayer adsorption of gemini surfactants onto the metal surface: (a) two hydrophilic ionic groups of gemini surfactant are adsorbed onto the metal surface site, (b) one hydrophilic group is adsorbed onto the surface site, while the other hydrophilic group is free in solution phase, and (c) both (a) and (b) co-exist. Visualization of the three sections is shown in Fig.(6). The first scenario (a) should be the main adsorption mechanism at low concentrations of gemini surfactants, and the gemini surfactant molecules tend to be adsorbed onto the steel surface at high concentrations according to the second scenario (b). But in fact, the third scenario should be more reasonable because of the interaction between molecules of gemini surfactant, which explains the increase in the inhibition efficiency accompanied with increasing the concentration of the tested surfactants.

3.5. Scanning electron microscopy (SEM)

Fig.(7-a) shows SEM image of carbon steel surface after immersion in sea water for 144 h in absence of inhibitor, while Figs.(7-b ,c and d) show SEM images of the steel specimen after immersion in sea water for the same time interval in presence of 6 m mol dm^{-3} of , 12-2-12 ,14-2-14, and 16-2-16 surfactants, respectively. The resulting scanning electron micrographs reveal that the surface was strongly damaged in absence of the inhibitor, but in presence of optimum concentration of surfactants there is much less damage of the surface. It is clear that, micrograph (7-d) shows smooth, well inhibited surface that confirms the good adsorption of 16-2-16 onto the used metal surface and, in turn, a high inhibition efficiency at this concentration.

4. Conclusions

1. All the studied cationic gemini surfactants exhibit S-shape adsorption isotherms with the formation of multilayer in case of 16-2-16.
2. Surface adsorption measurements, electrochemical studies, and weight loss measurements are in parallel to each other
3. The synthesized cationic gemini surfactants can be used as corrosion inhibitors for carbon steel in sea water. Their inhibiting properties increase with increasing the hydrocarbon chain length of the inhibitors according to order: 16-2-16 > 14-2-14 > 12-2-12.
4. Polarization measurements showed that the tested cationic gemini surfactants are mixed-type inhibitors.
5. The inhibition mechanism is attributed to the strong adsorption ability of the selected surfactants on carbon steel surface, forming a good protective layer, which isolates the surface from the aggressive environment.

Table (1) - The calculated and founded percentage values of C, H, and N for the tested gemini surfactants.

Elements / Surfactants	%C		%H		%N	
	Calculated	Found	Calculated	Found	Calculated	Found
12-2-12	58.56	57.75	10.75	10.79	5.08	5.76
14-2-14	60.91	59.11	11.05	11.50	4.18	4.42
16-2-16	62.81	61.98	11.29	11.53	3.86	3.99

Table (2) -Chemical composition of the used seawater

Element	Fe2+	Li+	Na+	K+	S^{-2}	Mg^{2+}	Cl^{-}	Ca^{2+}
Concentration (ppm)	0.74	3066.00	12325.63	394.91	3066.00	137.91	21110.00	459.00

Table (3) - Composition of the used low carbon steel alloy.

Element	Fe	C	S	Mn	P
Wt %	98.38	0.28	0.05	1.25	0.04

Table (٤) -Critical micelle concentration (CMC), excess surface concentration (Γ_{max}), and minimum surface area (A_{min}) of the used cationic gemini surfactants.

Parameters			
Surfactants	**CMC x 10^5, mol dm^{-3}**	**Γ_{max} x 10^{10}, Mol cm^{-2}**	**A_{min}, nm^2**
12-2-12	9.479	1.766	0.940
14-2-14	8.554	1.322	1.256
16-2-16	8.017	1.2578	1.78

Table (5) - Corrosion Rate (*K*) of carbon steel, Surface Coverage (□) and Corrosion inhibition Efficiency (η_w %) (obtained from weight loss) in absence and presence of different concentrations of surfactants after 144 h immersion in sea water at 25 °C .

Conc. m mol dm^{-3}	$C_{12-2-12}$			$C_{14-2-14}$			$C_{16-2-16}$		
	K mg cm h^{-1}	□	η_w%	*K* mg cm h^{-1}	□	η_w%	*K* mg cm h^{-1}	□	η_w%
0.0	0.198	-	-	0.198	-	-	0.198	-	-
0.04	0.072	0.4555	45.55	0.062	0.5254	51.54	0.039	0.7098	79.08
0.06	0.059	0.5230	52.30	0.062	0.6514	65.14	0.030	0.8831	88.31
0.08	0.049	0.6692	66.92	0.051	0.7115	71.15	0.029	0.8923	89.23
0.4	0.040	0.6825	68.25	0.0398	0.7625	76.25	0.025	0.9083	90.83
0.6	0.038	0.6890	68.90	0.033	0.7975	79.75	0.018	0.9292	92.92
0.8	0.036	0.7335	73.35	0.029	0.8231	82.31	0.012	0.9315	93.15
2	0.033	0.8012	80.12	0.022	0.8960	89.60	0.009	0.9535	95.35
4	0.029	0.8820	88.20	0.0135	0.9315	93.15	0.008	0.9615	96.15
6	0.019	0.9395	93.95	0.0112	0.9545	95.45	0.001	0.9885	98.85

Table (6) - Data obtained from potentiodynamic polarization measurements of carbon steel in sea water in absence and presence of various concentrations of the inhibitors at 25 °C.

Conc. m mol dm^{-3}	$C_{12-2-12}$			$C_{14-2-14}$			$C_{16-2-16}$		
	E_{corr} (mV)	i_{corr} (m A)	η_{pot} (%)	E_{corr} (mV)	i_{corr} (m A)	η_{pot} (%)	E_{corr} (mV)	i_{corr} (m A)	η_{pot} (%)
0.0	-975	0.686	-	-975	0.686	-	-975.4	0.686	-
0.04	-972	0.302	55.9	-970	0.281	59.0	-970	0.174	74.6
0.06	-971	0.248	63.9	-968	0.187	72.8	-969	0.075	89.0
0.08	-969	0.229	66.7	-967	0.128	81.3	-967	0.074	89.2
0.4	-968	0.222	67.7	-969	0.125	81.7	-964	0.062	90.9
0.6	-970	0.219	68.0	-965	0.118	82.7	-963	0.056	91.8
0.8	-966	0.204	70.2	-964	0.115	83.2	-961	0.048	92.9
2	-964	0.203	70.4	-963	0.111	83.8	-956	0.047	93.1
4	-963	0.194	71.7	-961	0.104	84.7	-953	0.044	93.5
6	-962	0.173	74.8	-959	0.087	87.2	-950	0.040	94.0

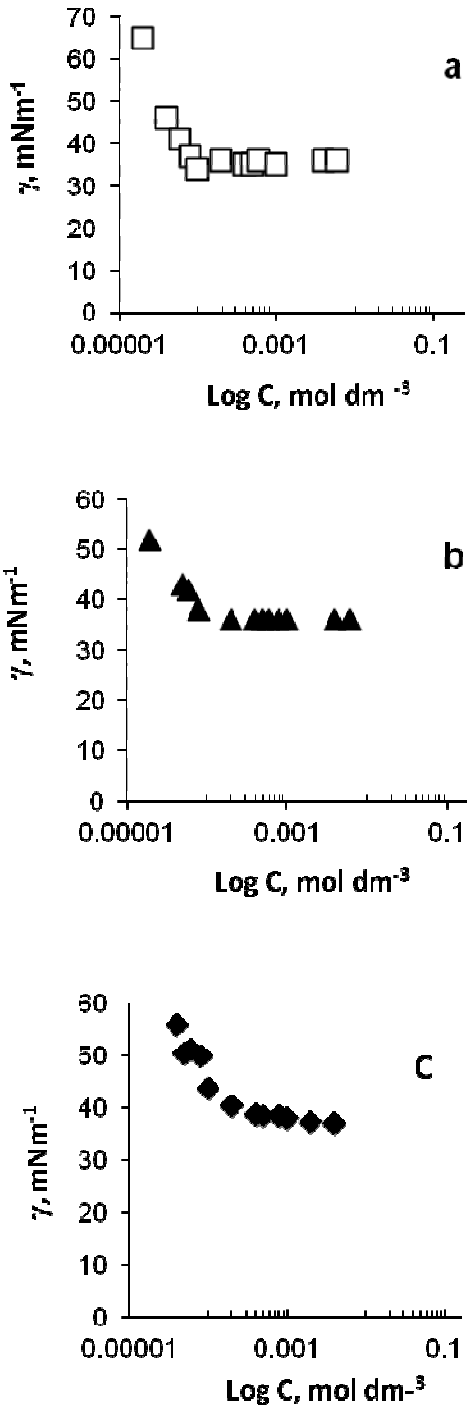


Fig (1)- Surface tension vs log of molar surfactant concentration for the tested cationic gemini surfactants (a)12-2-12, (b) 14-2-14 and (c) 16-2-16.

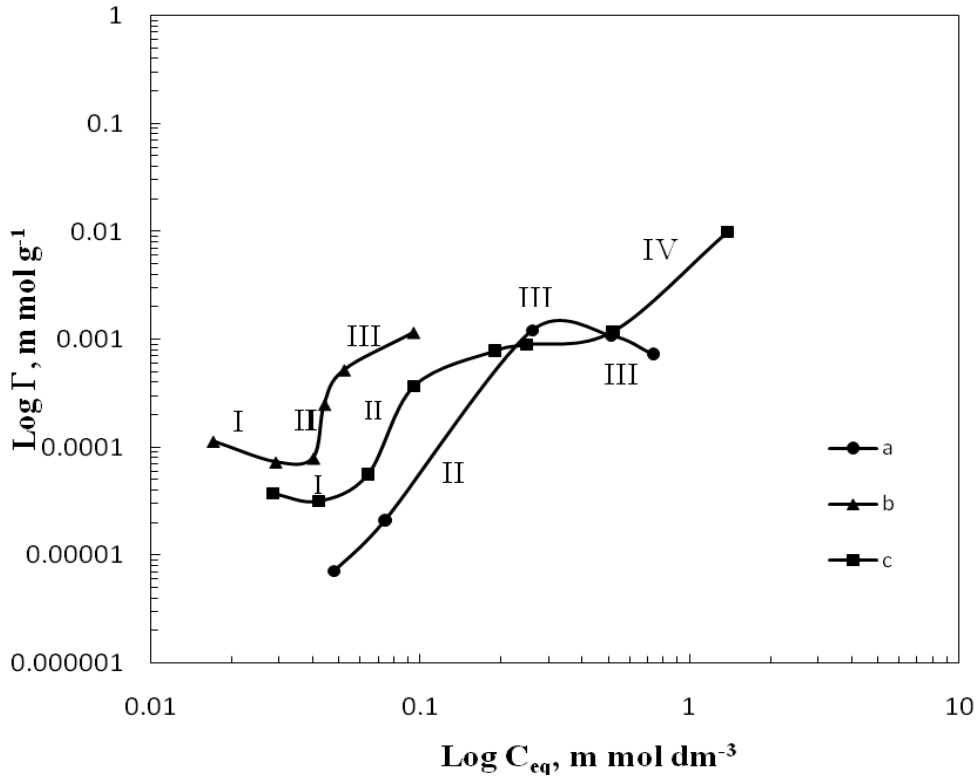


Fig (2) – Adsorption isotherms of tested cationic Gemini surfactants from sea water onto carbon steel surface, (a) 12-2-12, (b) 14-2-14, (c) 16-2-16.

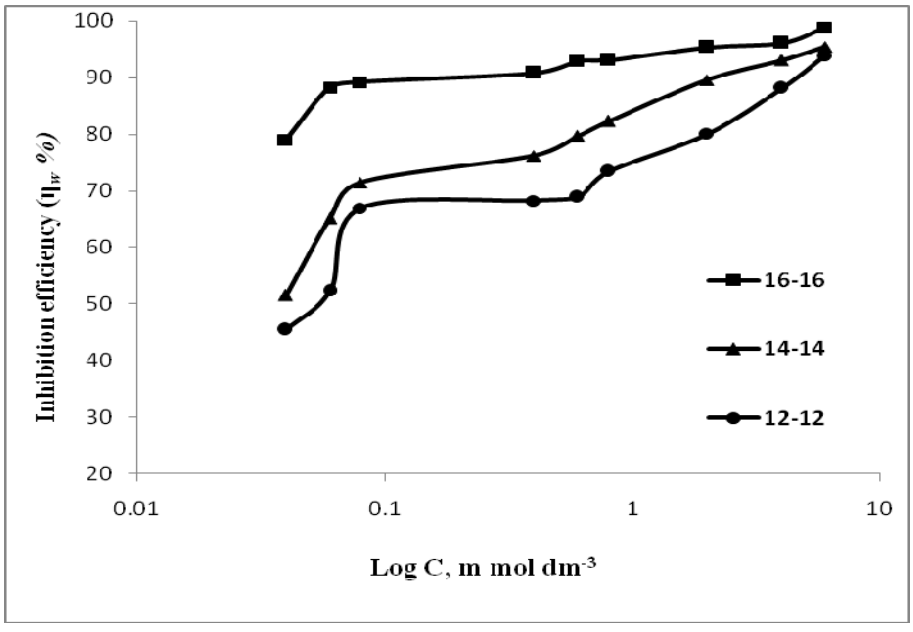


Fig .(3)- inhibition efficiency of different tested surfactants (obtained from weight loss) as a function of surfactant concentration.

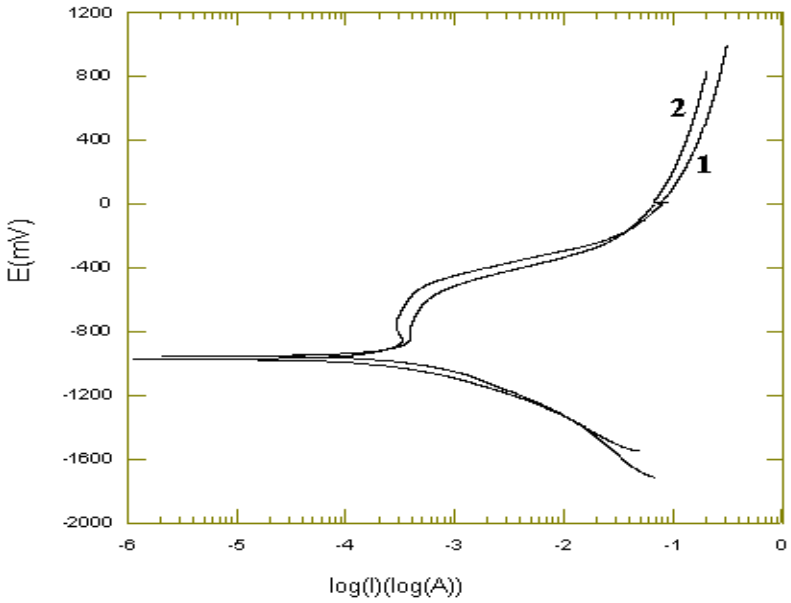


Fig .(4)- Potentiodynamic polarization curves of carbon steel in sea water ,(1) in absence of inhibitor ,(2) in presence of optimum concentration (6 m mol dm^{-3}) of 16-2-16 cationic gemini surfactant.

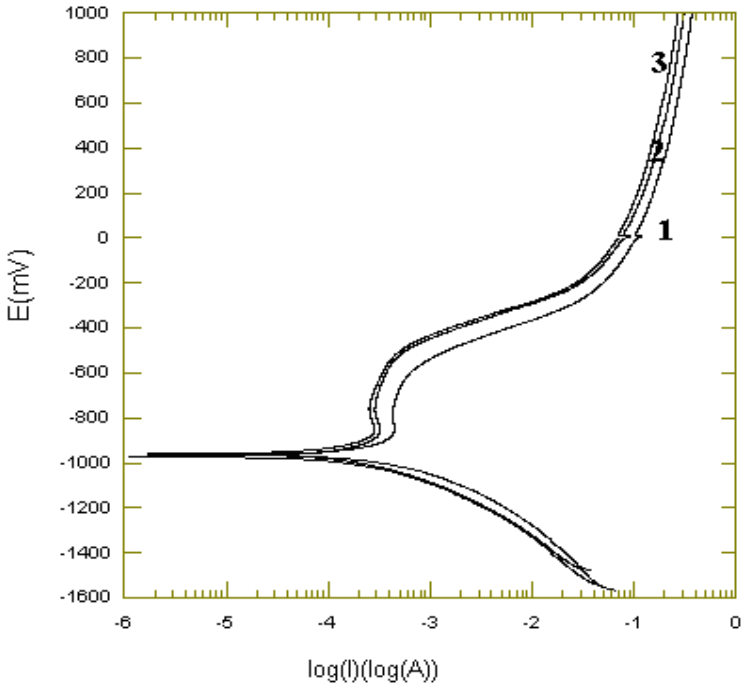


Fig.(5):Potentiodynamic polarization curves of carbon steel in seawater , (1) in absence of inhibitor and in presence of optimum concentration(6 m mol dm^{-3}) of (2) 12-2-12 and (3) 14-2-14 cationic gemini surfactants.

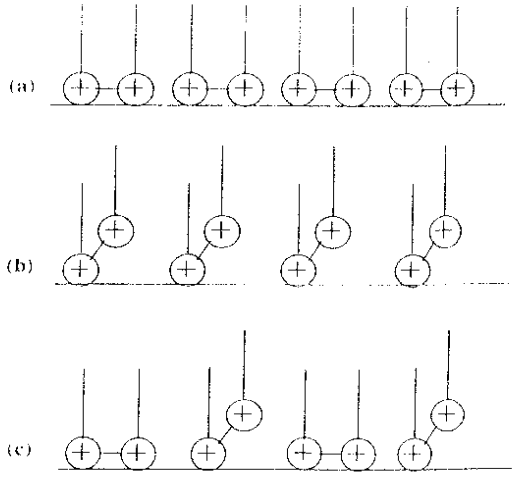
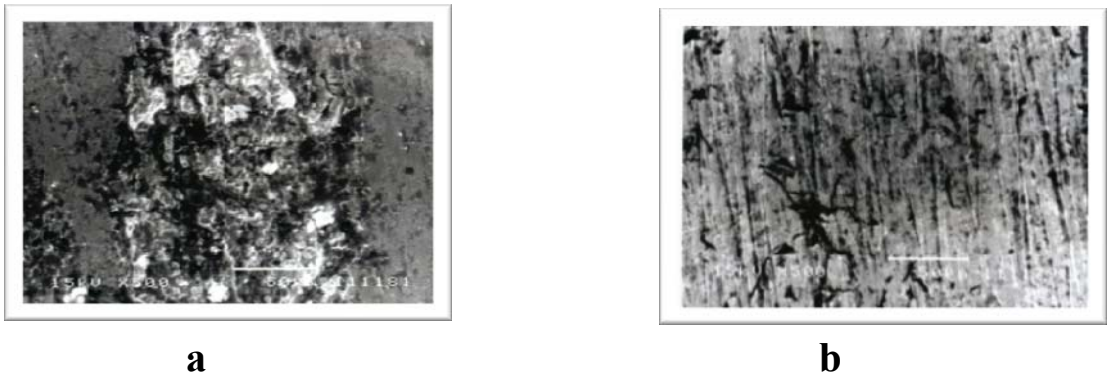
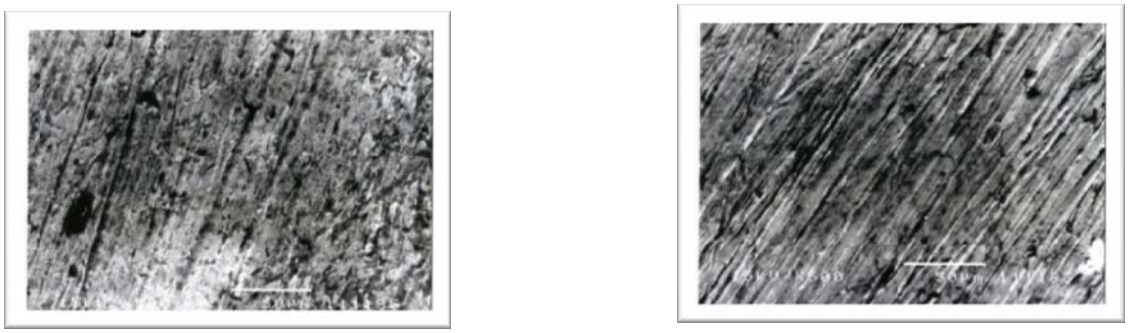


Fig. (6) – Schematics for monolayer adsorption of Gemini surfactant onto carbon steel surface.



a **b**



C **d**

Fig. (7) – Scanning electron micrographs of carbon steel surface after immersion for 144 h in free inhibitor sea water (a) 12-2-12 (b) 14-2-14 (c) and 16-2-16 (d) gemini surfactants .

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Prophylactic and therapeutic evaluation of the phytobiotic (Orego-stim)® in chicken experimentally infected with *E. coli*

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Abstract: The prophylactic and therapeutic effects of the phytobiotic (Orego-stim)® was evaluated in chicken experimentally infected with Novobiocin marked *E. coli O78*. Enrofloxacin (Opitryl)® was used as a standard. The obtained results demonstrated that, birds prophylactically received Orego-stim® showed more favorable clinical signs, mortality rate, P.M. lesions, recovery rate, bacterial reisolation results and growth performance. Both cellular and humeral immunity were enhanced. A decrease in the mean values of serum ALT & AST ,albumin, uric acid and creatinine levels were recorded that may provide evidence for the hepato and renoprotective effects of the essential oils. It could be concluded that, Orego-stim® can be considered a promising mixture of essential oils due to its high efficacy (growth performance, antibacterial and immunomodulating effects) and positive impact on both liver and kidney functions. The study highly recommends the use of Orego-stim® as a prophylactic agent in dealing with *E. coli* infection in chicken however, its concurrent administration with enrofloxacin in treatment of such case revealed the most favorable outcomes.

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Key words: Orego-stim®, essential oils, *E. coli*, efficacy, side effects, chicken

1. Introduction:

In the last few years, frequent outbreaks of resistant bacterial strains in field settings occurred and claims regarding the hazard of multiple resistance to antibiotics used in human and animal medicine increased due to indiscriminate use of commercial antimicrobial drugs/chemical commonly used in the treatment of infectious diseases or as food additives ***(Davis, 1994; Service,1995).***

These concepts have led the European Union to ban the use of most of these additives in poultry feeds. For this reason, many studies have recently been undertaken investigating the use of novel yet promising natural feed-additives "organic acids, probiotics, prebiotics, plant extracts, enzymes and essential oils" ***(Schwarz et al., 2001)***. Herbs, spices and various plant extracts have received increased attention as possible replacements for antibiotic growth promoters ***(Burt, 2004 and Peschel et al., 2006)***.

In this context, aromatic plants, especially species of Origanum genus, have emerged as effective compounds seeking microbiological safety represented by phytobiotic ***(Ipek et al., 2005 and Souza et al., 2007)***.

The genus Origanum (Family: Lamiaceae) comprises about 38 species widespread all over the world. Among them, *Origanum vulgare is* an endemic spontaneous plant, growing in North Africa and used as a medicinal plant against whooping cough, cough, fever and bronchitis ***(Baba Aissa, 1991).***

The notion that, *Origanum species* has antibacterial, antifungal, insecticidal, antioxidant and anti-carcinogenic activities motivated the study of the biological activities of their essential oil contents ***(Ipek et al., 2005)***. In fact, purified essential oils and their fractions; carvacrol, thymol, limonene and cineole; have shown extensively to have antimicrobial properties *in vitro* ***(Ultee et al., 2002 and Faleiro et al., 2003).*** Mode of their antimicrobial action consists of interactions with cell membranes changing the permeability of cations such as H+ and K+ ***(Ultee et al., 1999).***

Orego-stim® is a phytobiotic containing Oreganum aetheroleum as active substance. Oreganum aetheroleum is oregano etheric oil obtained by steam distillation of the leaves and flowers of the plant *Origanum vulgare ssp. hirtum* that has been demonstrated to possess wide-spectrum antibacterial activities and has been extensively used by people for the treatment of bacillary dysentery, enteritis and cholera etc. The principal ingredients are phenolic derivatives, such as carvacrol and thyme camphor, which have strong antibacterial effects ***(Hammer et al., 1999).***

In this spirit, then, the current work was designed to evaluate the prophylactic and therapeutic effects of the phytobiotic (Orego-stim®) in chicken experimentally infected with *E. coli*

2- Material and Methods

2.1. Agents:

2.1.1. Orego-stim®:

Orego-stim®; a product of Meriden Animal Health Co. – UK; is a phytobiotic used as a natural feed/drinking water additive. It contains Oreganum aetheroleum as active substance. Oreganum aetheroleum is oregano etheric oil obtained by steam distillation of the leaves and flowers of the plant *Origanum vulgare ssp. hirtum* and contains many essential oils, mainly *carvacrol* 81.89%, *y- terpinrnr* 5.1%, *p- cymene* 3.76% and *thymol* 2.12%. It was given as oral solution at a dose of 0.3 ml/ liter.

2.1.2. Antibiotic:

Enrofloxacin (Opitryl)®; a product of El-Obour Modern Pharmaceutical Industries Co., Egypt. Each 100ml contains 10gm enrofloxacin.

It was given as a 10% oral solution in the recommended therapeutic dose of 10 mg/kg of bird's body weight (1 ml /2 liter) for 5 successive days ***(Luke et al., 2006).***

2.1.3. Bacteria

*E. coli O*78 Kindly supplied from the Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Zagazig University.

2.1.3.1. Preparation of bacterial cultures:

*E. coli O*78 was reconstituted in 5ml nutrient broth and incubated at 37°C for 24hrs, then sub-cultured on MacConkey's agar and incubated at 37°C for 24hrs.

2.1.3.2. Preparation of Novobiocin marked strains:

E. coli O78 resistant strains was prepared by sub-passage of the micro-organism on media contained 1gm Novobiocin /liter after several sub-passage on graded levels of the Novobiocin ***(Barnhart et al., 1999)***.

2.1.3.3. Bacterial titration:

Ten fold dilutions were prepared from 24hrs cultures on peptone water to obtain 10^8 CFU/ml to be used for inoculation of chicks according to ***Sambrook et al., 1989***.

2.2. Experimental chicks:

Sixty, one day old, commercial (Hubbard) broiler chicks obtained from El-Banna Poultry Company were used for the experiment. The chicks were floor reared and fed on a balanced commercial ration free from antimicrobial agents.

2.3. Experimental design:

On the 1st day of age, chicks were divided into 6 equal groups; 10 each.

The 1st group : was not inoculated and left as a control (-ve control).

From the 2nd group through the 6th group, Novobiocin marked *E. coli O78* was inoculated I/nasal at approximately $1x10^8$ CFU /ml on the 5th day of age.

The 2nd group: was kept as +ve control (infected, non-treated).

The 3rd group: was inoculated, treated with the recommended dose of Orego- stim®; 0.3ml / liter orally for 5 successive days.

The 4th group: was inoculated, treated with the therapeutic dose of enrofloxacin (Opitryl)®; 10 mg/kg of body weight of birds (1 ml / 2 liter) orally for 5 successive days.

The 5th group: was inoculated, treated with both Orego-stim® and enrofloxacin (Opitryl)®; at doses as mentioned in the 3rd and 4th groups respectively for 5 successive days.

The 6th group: received Orego-stim® from the 1st day of age throughout the experimental period and inoculated with *E. coli O78* I/nasal at approximately $1x10^8$ CFU /ml on the 5th day of age.

2.4. Samples collection and preservation:

On the 15th, 22nd and 29th and 36th days of age, five chickens from each group were used in each collection .Two blood samples, 2.5 ml each were collected from the wing vein of each chick. The first blood sample was collected in test tube containing 50 I.U. /ml blood heparin as anticoagulant to determine the phagocytic activity and phagocytic index. The second blood sample was collected in a centrifuge tube and left to clot then centrifuged at 2000 rpm for 10 minutes to allow serum separation which then aspirated into cryovials and stored at -20°C for humeral immunity investigation and liver & kidney function tests.

2.5. Analysis:

2.5. 1. Evaluation of Orego-stim® efficacy:

2.5. 1. 1. Clinical signs, P.M. lesions, Mortality rate and bacterial reisolation:

All groups were kept under observation for a week post inoculation, clinical signs; postmortem lesions and mortality rate were recorded.

On the 16th day of age, dropping samples were collected under aseptic conditions for bacteriological investigation. One gram of the sample was weighted and placed in a sterilized glass tube. The initial dilution was made by adding 9 volumes of sterile saline (Nacl solution 0.9%). Further serial 10 fold dilutions using sterile saline solution were made. Following dilution, 0.01ml of the suspension from the dilutions, including the initial dilution, were taken and spread on MacConkey's agar containing 25 µg Novobiocin for reisolation of *E. coli* **(*Edao et al., 1998*).**

2.5.1.2. Growth performance evaluation:

From the 1st day of age throughout the experimental period, body weight (B.W.), feed intake (F.I.) and food conversion rate (F.C.R.) for each group were recorded weekly

2.5.1.3. Immunological response evaluation:

2.5.1.3.1. Cellular immunity:

By determination of Phagocytic activity and phagocytic index ***(Kawahara et al., 1991).***

Phagocytic activity (PA) = percentage of phagocytic cells containing yeast cells.

Phagocytic index (PI) = Number of yeast cells phagocytozed / Number of counted phagocytic cells

2.5.1.3.2. Humeral immunity:

By Quantitative estimation of serum IgM ***(Young, 1997)*** and IgG ***(Friedman and young, 1997).***

2.5.2. Evaluation of Orego-stim® side effects:

2.5.2.1. Effect on Liver functions:

By quantitative estimation of serum total proteins and albumen ***(Weichselbaum, 1946 & Doumas, 1971)*** and liver enzyme activities (ALT & AST) ***(Reitman and Frankel, 1957)***.

5.2.2.2. Effect on kidney functions:

By quantitative estimation of serum uric acid ***(Haisman and Muller, 1977)*** and creatinine ***(young et al., 1975***).

2.5.3. Statistical Analysis:

The obtained data were analyzed statistically using an ANOVA test according to ***(SPSS Win, 1995).***

3- Results

3.1. Evaluation of Orego-stim® efficacy:

3.1.1. Clinical signs, P.M. lesions, Mortality rate and bacterial reisolation:

Birds belonged to groups (2); inoculated with Novobiocin marked *E. coli O*78 and received no treatment; showed the severest *E.coli* infection signs compared with other groups. Signs included coughing, sneezing, snicks, rales, ocular and nasal discharge. Diarrhea and dehydration were also noted on clinical examination. Feed intake and growth rate were reduced compared with control –ve chicks (group 1). Occasional birds had a hypopyon and/or hyphema, usually in one eye, which was blind (Panophthalmitis). Mortality rate was 10%. At necropsy, the most pronounced lesions were thickened air sacs with caseous exudates, adhesive fibrinous pericarditis, fibrinous perihepatitis and enteritis with excessive fluid in the intestine. Marked *E. coli O*78 was reisolated from 60% of the birds on the 16th day of age.

Birds belonged to groups 3, 4, 5, 6 were inoculated with Novobiocin marked *E. coli O*78 and treated with Orego-stim® only, Enrofloxacin only, both Orego-stim® and Enrofloxacin, and Orego-stim® from the 1st day of age throughout the experimental period respectively showed the same clinical signs previously recorded in group 2 but with variable milder degrees with special reference to group 6 which displayed the mildest symptoms. Recovery rate was 70%,80%,100% and 90% respectively and marked *E. coli O*78 was reisolated from 30% ,20%, zero% and 10% of the birds respectively on the 16th day of age.

3.1.2. Growth performance evaluation:

Birds belonged to groups (2) demonstrated significant decrease ($P<0.05$) in the mean values of body weight (B.W.) allover the experimental period compared with the –ve control group (Table 1).

Chicks in groups (4, 5, 6) showed significant increase ($P<0.05$) in the mean values of body weight (B.W.) on the 7th, 14th, 35th and 42nd days of age compared with the group (2).

Birds belonged to group (6) displayed significant increase ($P<0.05$) in the mean values of body weight (B.W.) on the 28th, 35th and 42nd days of age compared with the group (3) and birds belong to groups (5) demonstrated a significant increase ($P<0.05$) in the mean values of body weight (B.W.) on the 35th and 42nd day of age compared with the group (4).

The results showed that, chicks in groups (2) showed significant increase ($P<0.05$) in the mean values of feed intake (F.I.) on the 14th ,21st ,28th and 35th days of age compared with the –ve control group (table 1)

Birds in group (6) showed significant decrease($P<0.05$) in the mean values of F.I. on the 7th ,21st ,28th and 35th days of age compared with the

group (3) while chicks in group (5) showed significant increase ($P<0.05$) in the mean values of F.I on the 21st and 28th days of age compared with the group (4).

The obtained results clearly demonstrated that, (group 2) displayed significant increase ($P<0.05$) in the mean values of food conversion rate (F.C.R.) allover the experimental period compared with the control –ve group while chicks in groups (3, 4, 5, 6) showed significant decrease ($P<0.05$) in the mean values of F.C.R. on 21st, 28th, 35th and 42nd day of age compared with the group (2) (Table 1)

Also, birds in group (6) displayed significant decrease ($P<0.05$) in the mean values of F.C.R. on 7th, 28th, 35th and 42nd day of age compared with the group (3) while chicks belonged to group (5) showed significant decrease ($P<0.05$) in the mean values of F.C.R. on 35th and 42nd day of age compared with the group (4).

3.1.3. Immunological response evaluation:

3.1.3.1. Phagocytic activity and phagocytic index:

The results showed that, birds belonged to group (2) demonstrated a significant increase ($P<0.05$) in the mean values of phagocytic activity & phagocytic index on the 15th and 22nd day of age compared with the –ve control group (Table 2).

Birds belonged to groups (3, 5, 6) demonstrated significant increase ($P<0.05$) in the mean values of phagocytic activity & phagocytic index on the 15th, 22nd, 29th and 36th day of age compared with the +ve control group .

On the 22nd, 29th and 36th day of age, birds of group (4) showed significant decrease ($P<0.05$) in the mean values of phagocytic index compared with group (2) values while groups (6&5) showed significant increase in the mean values of phagocytic activity & phagocytic index compared with groups (3 &4) values respectively on the 15th, 29th and 36th day of age.

3.1.3. 2. Quantitative estimation of serum IgM and IgG :

The results showed that, birds belonged to groups (2) demonstrated a significant increase ($P<0.05$) in the mean values of IgM & IgG compared with the –ve control group (Table 2).

Birds belonged to groups (3, 5, 6) demonstrated significant decrease ($P<0.05$) in the mean values of IgM compared with the +ve control group while birds belong to groups (3, 6) showed significant increase ($p<0.05$) in the mean values of IgM compared with control –ve group .There were non significant changes in the mean values of IgG in the birds of groups (3, 4, 5, 6) compared with –ve control group.

3.2. Evaluation of Orego-stim® side effects:

3.2.1. Effect on Liver functions:

The results revealed that, birds belonged to groups (2) demonstrated significant increase ($P<0.05$) in the mean values of total proteins on the 15th day of age compared with the control –ve group (Table 3).

Birds belonged to groups (3, 5, 6) demonstrated significant increase ($P<0.05$) in the mean values of total proteins on the 15th day of age compared with the -ve control group. Chicks in group (5) showed significant increase ($P<0.05$) in the mean values of total proteins on the 15th day of age compared with group (4).

Concerning albumin level, birds in group (2) demonstrated significant decrease ($P<0.05$) in the mean values of albumin on the 15th day of age compared with the -ve control group. Chicks in groups (3, 4, 5, 6) showed significant increase ($P<0.05$) in the mean values of albumin on the 15th day of age compared with group (2) and non significant changes compared with control –ve group .

The results clearly demonstrated that, birds in group (2) showed significant increase ($P<0.05$) in the mean values of serum ALT & AST levels on the 15th and 22nd day of age compared with the -ve control group . Chicks in groups (3, 4, 5, 6) showed significant decrease ($P<0.05$) in the mean values of serum ALT & AST levels on the 15th and 22nd day of age compared with group (2) .

Birds belonged to groups (6) demonstrated significant decrease ($P<0.05$) in the mean values of serum ALT & AST levels on the 15th and 22nd day of age compared with group (3) and chicks in group (5) showed significant decrease ($P<0.05$) in the mean values of serum AST level on the 22nd day of age compared with group (4).

3.2.2. Effect on Kidney functions:

The obtained results clearly demonstrated that, on the 15th and 22nd day of age, control +ve chicks (group 2) displayed significant increase ($P<0.05$) in the mean values of uric acid & creatinine compared with the control –ve group (Table 4).

Birds belonged to groups (3, 4, 5, 6) demonstrated significant decrease ($P<0.05$) in the mean values of uric acid and creatinine compared with the +ve control group. Chicks in group (6) displayed significant decrease ($P<0.05$) in the mean values of uric acid & creatinine compared with those in group (3) and birds of group (5) showed significant decrease ($P<0.05$) in the mean values of uric acid & creatinine compared with group (4).

4- Discussion

The present study was designed to evaluate the prophylactic and therapeutic effects of the phytobiotic mixture of essential oils (Orego-stim®) in chicken experimentally infected with *E. coli*.

The results clearly demonstrated that birds received Orego-stim® pre infection (group 6) showed more favorable clinical signs, mortality rate, P.M. lesions , recovery rate and bacterial reisolation results compared with those treated with it post infection (group 3) or infected and none treated at all (group 2).

Orego-stim® is a phytobiotic contains Oreganum aetheroleum as active substance. Oreganum aetheroleum is oregano etheric oil obtained by steam distillation of the leaves and flowers of the plant *Origanum vulgare ssp. hirtum* and contains many essential oils, mainly *carvacrol* 81.89%, *y- terpinrnr* 5.1%, *p- cymene* 3.76% and *thymol* 2.12% to which the broad spectrum antimicrobial activity of *Origanum* oil is attributed ***(Knobloch et al., 1989; Bendahou et al., 2008)***. Its antimicrobial activities against bacteria, yeast and fungi such as, *E. coli*, *Pseudomonas aeruginosa*, *Staph. aureus*, *Enterococcus hirae*, *Candida albicans* and *Candida tropicalis* was proved ***(Sari et al., 2006)*** and recognized since antiquity ***(Leung and Fostere , 1996)***.

It has been suggested that phenolic derivatives can cause membrane-disturbing activities that change the permeability of cations such as H^+ and K^+ (***Ultee et al., 1999; Ipek et al., 2005)***. Their hydrophobic characters enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable ***(Tepe et al., 2004).***

Hydrophobicity of carvacrol, the essential oil of the highest amount in *O. vulgare*, and its mode of action suggest the partition in the cytoplasm membrane ***(Ultee et al., 2002)***. Exposure of bacterial cells to carvacrol increases the membrane fluidity causing leakage of protons and potassium ions, leading to a decrease in pH gradient across the cytoplasm membrane, a collapse of the membrane potential, an inhibition of ATP synthesis and ultimately the cell death ***(Ultee et al., 1998; Ultee et al., 2000)***. Addition of carvarcrol to bacterial cell suspensions causes a dose-related extension of the lag-phase, a lower maximum specific growth rate, and a lower final population density ***(Lambert et al., 2001)***.

Sublethal injury of microbial cell membrane provided by subinhibitory concentrations of antimicrobial compounds may alter their permeability and affect the ability of the membrane to osmoregulate the cell adequately or to exclude toxic material ***(Carson et al., 2002)***. The loss of salty tolerance could reveal membrane damage in sublethally injured *Staph. aureus* cells caused by *O. vulgare* essential oil.

Concerning the effect of Orego-stim® on F.I., B.W. and F.C.R., our results revealed that, birds belonged to groups (6) and (5) showed more favorable results compared with those belonged to groups (3) and (4) respectively.

Several researchers showed that, supplementation of some essential oils stimulates the animal digestive systems ***(Ciftci et al., 2005)*** to increase production of digestive enzymes and improve utilization of digestive products through enhanced liver functions ***(Hermandez et al., 2004)***. Thus**,** improves feed intake, feed conversion ratio ***(Alcicek et al., 2003; Halle et al., 2004; Cross et al., 2007)***, promoting a better sedimentation of muscle protein ***(Zheng et al., 2009)***, and so improves the live body weight ***(Denli and Uluocak, 2004)***.

On the other hand, it was found that the probable beneficial effect produced by thymol essential oil was its nutrient digestibility ***(Langhout, 2000).*** Likewise ***Hernandez et al. (2004)*** suggested that plant extract supplementation improved apparent whole- tract and ileal digestibility of nutrients.

In addition to the antimicrobial activity of essential oils ***(Valero and Salmeron, 2003)*** they posses biological activities such as that of antioxidants ***(Miura et al., 2002; Zheng et al., 2009)*** and as hypocholesterolemics ***(Craig, 1999)*** that enhances growth performances.

Our findings fit in with the results obtained by ***Alcicek et al. (2003)*** who found that, supplementation of Orego-stim® improved the F.C.R. in broilers which may attributed to the stimulation of digestion induced by Orego-stim® .Other study reported that, essential oils derived from different aromatic plants improved weight gain and F.C.R. ***(Giannenas et al., 2003; Jamroz et al., 2005).***

Regarding the effect of Orego-stim® on the immune response, the results showed that, birds belonged to groups (6, 5) showed significant increase in the mean values of phagocytic activity & phagocytic index compared with those belonged to groups (3, 4) respectively on the 15th, 29th and 36th day of age and birds belonged to groups (3, 6) showed significant increase in the mean values of IgM compared with control –ve group.

As a matter of fact, however plants and their bioactive components, when known, are very diverse and their potential to enhance animal health and immunity has only been scarcely evaluated *in vivo*. Mixtures of essential oils based on thymol and carvacrol, whose major sources are thyme and oregano respectively ***(Burt, 2004)*** seem promising

due to their potential immunomodulatory properties ***(Woollard et al., 2007; Gabor et al., 2010)***.

It was found that, extract of *Origanum vulgare*, enriched with thymol and carvacrol in similar proportions, was reported to protect animals from diseases. That health benefit was associated with an increased proportion of CD4+, CD8+ and double positive T cells in peripheral blood and mesenteric lymph nodes ***(Walter and Bilkei, 2004).*** Thymol used alone enhances total IgA and IgM serum levels and exhibits some local anti-inflammatory properties, as indicated by a reduction in TNF-mRNA in the stomach of post-weaned pigs ***(Trevisi et al., 2007).***

In this frame of references, it is fitting to mention that, neutrophils are highly specialized for their primary function, that is, recognition, phagocytosis and destruction of microorganisms. The interaction between microorganisms and neutrophils induces complex and concerted structural and metabolic alterations of the neutrophils, essential for normal function ***(Bjerknes, 1998)***.

Table (1): Effects of oral administration of Orego-stim®; 0.3ml / liter and Enrofloxacin (Opitryl®; 10 mg/kg kg B.W. of birds (1 ml / 2 liter) on growth performance (Body weight "B.W.", Feed Intake "F.I." and Food conversion rate " F.C.R.") in chicks experimentally infected with *E. coli*.

Age day	Group 1			Group 2			Group 3			Group 4			Group 5			Group 6		
	B.W. (gm)	F.I. (gm)	F.C.R.	B.W. (gm)	F.I. (gm)	F.C.R.	B.W. (gm)	F.I. (gm)	F.C.R.	B.W. (gm)	F.I. (gm)	F.C.R.	B.W. (gm)	F.I. (gm)	F.C.R.	B.W. (gm)	F.I. (gm)	F.C.R.
7	135.0± 2.88^{A}	123.0± 1.73^{C}	0.91± 0.02^{C}	110.0± 2.88^{B}	109.0± 2.30^{D}	0.99± 0.005^{ABC}	136.6± 3.17^{A}	145.0± 2.88^{A}	1.06± 0.005^{A}	129.0± 5.19^{A}	130.0± 5.77^{BC}	1.006± 0.003^{ABC}	129.0± 10.96^{A}	134.0± 2.30^{B}	1.050± 0.08^{AB}	128.0± 4.61^{A}	120.0± 2.88^{C}	0.93± 0.008^{BC}
14	323.8± 1.73^{BC}	380.0± 2.30^{C}	1.17± 0.005^{C}	288.0± 1.73^{D}	403.2± 1.84^{B}	1.36± 0.03^{B}	317.5± 4.33^{C}	305.0± 2.88^{D}	0.96± 0.005^{D}	337.0± 4.04^{A}	460.0± 2.88^{A}	1.36± 0.01^{B}	330.5± 3.17^{AB}	470.0± 5.77^{A}	1.41± 0.01^{A}	315.5± 2.74^{C}	305.0± 2.88^{D}	0.96± 0.003^{D}
21	686.0± 1.15^{A}	841.0± 1.73^{C}	1.22± 0.003^{D}	623.8± 1.73^{C}	1035.5± 2.88^{A}	1.65± 0.003^{A}	690.0± 2.88^{A}	755.0± 1.73^{E}	1.09± 0.003^{E}	644.7± 1.90^{B}	870.0± 5.77^{C}	1.34± 0.01^{C}	646.2± 3.40^{B}	890.0± 2.30^{B}	1.37± 0.006^{B}	630.0± 5.77^{C}	680.0± 8.66^{F}	1.07± 0.01^{E}
28	913.3± 1.55^{A}	1530± 4.61^{B}	1.67± 0.003^{B}	872.2± 4.15^{AB}	2023.7± 5.05^{A}	2.32± 0.005^{A}	850.0± 5.77^{D}	1363± 1.73^{E}	1.60± 0.011^{C}	856.3± 3.29^{BC}	1438± 4.61^{D}	1.68± 0.005^{B}	878.5± 4.90^{B}	1460± 2.88^{C}	1.65± 0.008^{B}	874.6± 10.91^{BC}	1293± 1.73^{F}	1.47± 0.01^{D}
35	1283.3± 1.70^{B}	2752.2± 1.30^{B}	2.14± 0.003^{C}	1137.8± 4.5^{E}	2958± 4.56^{A}	2.59± 0.008^{A}	1146.0± 3.46^{E}	2585± 2.88^{E}	2.25± 0.01^{B}	1189.0± 5.19^{D}	2690± 5.77^{C}	2.26± 0.005^{B}	1312.0± 1.15^{A}	2665± 2.88^{D}	2.03± 0.003^{D}	1239.0± 5.19^{C}	2495± 2.88^{F}	2.01± 0.005^{E}
42	1650.0± 5.77^{A}	4139.7± 5.08^{A}	2.50± 0.008^{E}	1400.0± 2.88^{E}	4032± 4.04^{B}	2.88± 0.005^{A}	1380.0± 11.54^{F}	3680± 5.77^{E}	2.66± 0.02^{C}	1420.0± 2.88^{D}	3865± 2.30^{C}	2.72± 0.005^{B}	1490.0± 1.15^{B}	3765± 2.88^{D}	2.52± 0.003^{DE}	1444.4± 2.66^{C}	3756± 0.63^{D}	2.56± 0.03^{D}

Group (1) Non infected, non treated chicks (-ve control) Group (2) *E. coli* infected chicks (+ve control)

Group (3) Infected chicks & treated with the Orego-stim® Group (4) Infected chicks& treated with the therapeutic dose of enrofloxacin (Opitryl)®

Group (5) Infected chicks& treated with Orego-stim® and therapeutic dose of enrofloxacin (Opitryl)®

Group (6) Chicks given Orego-stim® from the 1st day old throughout the experimental period and infected with *E. coli* on the 5th day of age

Means carrying different superscripts in the same column are significant at $p< 0.05$

In this context, ***Toshifumi et al. (1995)*** determined phagocyte percentage, phagocyte index as parameters of phagocyte function. They recorded that neutrophilic activity is less active than that of opsonin. These parameters are significantly stimulated by Thymol, both on the level of neutrophilic phagocyte function and the opsonin but it was more predominant as regards the neutrophilic phagocyte activity. This finding is explained by ***Farinacci et al. (2008)*** who mentioned that *Origanum vulgaris* modulate the neutrophils immune function.

The current work demonstrated that, chicken in groups (3, 4, 5, and 6) showed significant decrease in the mean values of serum ALT & AST , uric acid and creatinine levels compared with group (2). Birds belonged to groups (6) demonstrated significant decrease in the mean values of serum ALT & AST, uric acid and creatinine levels compared with group (3) and chicks in group (5) showed significant decrease in the mean values of serum AST; uric acid and creatinine levels compared with group (4).

Generally AST and ALT considered as liver enzyme which increased with liver damage (heptatocellular degeneration), so the decrease in AST and ALT may provide evidence for the occurrence of hepatoprotective effect of the essential oils ***(Hermandez et al., 2004)***.

Table 2: Effects of oral administration of Orego-stim®; 0.3ml / liter and Enrofloxacin (Opitryl)®; 10 mg/kg B.W. of birds (1 ml / 2 liter) on Phagocytic (activity & index) and serum levels of IgM & IgG (mg/dl) in chicks experimentally infected with *E. coli*.

Groups	Phagocytic activity (%)				Phagocytic Index (P.I)				IgM	IgG
	Age in days				Age in days				Age in days	
	15th	22nd	29th	36th	15th	22nd	29th	36th	15th	22nd
1	8.82± 0.3^{E}	8.65± 0.41^{E}	8.08± 0.42^{D}	7.63± 0.15^{C}	0.92± 0.02^{D}	1.01± 0.06^{E}	0.91± 0.03^{D}	0.86± 0.03^{CD}	5.06± 0.52^{CD}	42.9± 1.0^{BC}
2	12.68± 0.59^{D}	11.44± 0.35^{D}	8.49± 0.55^{D}	7.03± 0.07^{C}	1.40± 0.12^{C}	1.26± 0.04^{C}	0.84± 0.06^{D}	0.74± 0.01^{D}	13.43± 0.61^{A}	49.2± 0.43^{A}
3	20.86± 2.43^{B}	23.33± 0.59^{B}	23.65± 1.2^{B}	19.68± 0.92^{B}	1.75± 0.05^{B}	1.86± 0.08^{AB}	1.81± 0.04^{B}	1.51± 0.03^{B}	10.13± 0.18B	43.4± 1.2^{BC}
4	9.08± 0.63^{DE}	8.28± 0.57^{DE}	8.57± 0.3^{D}	8.68± 0.32^{C}	1.23± 0.13^{C}	1.19± 0.16^{D}	1.43± 0.10^{C}	1.10± 0.08^{C}	4.26± 0.86^{D}	39.1± 0.52^{C}
5	16.51± 0.68^{C}	16.78± 0.21^{C}	17.63± 0.57^{C}	17.95± 1.03^{B}	1.46± 0.10^{C}	1.57± 0.07^{B}	1.59± 0.07^{BC}	1.48± 0.01^{B}	6.10± 0.75^{C}	42.8± 1.9^{BC}
6	29.34± 1.24^{A}	29.66± 0.36^{A}	29.9± 0.84^{A}	30.64± 1.23^{A}	2.07± 0.06^{A}	2.09± 0.12^{A}	2.25± 0.16^{A}	2.43± 0.23^{A}	9.06± 0.40^{AB}	46.8± 3.1^{AB}

Group (1) Non infected, non treated chicks (-ve control) Group (2) *E. coli* infected chicks (+ve control)

Group (3) Infected chicks & treated with the Orego-stim® Group (4) Infected chicks& treated with the therapeutic dose of enrofloxacin (Opitryl)®

Group (5) Infected chicks& treated with Orego-stim® and therapeutic dose of enrofloxacin (Opitryl)®

Group (6) Chicks given Orego-stim® from the 1st day old throughout the experimental period and infected with E. coli on the 5th day of age

Means carrying different superscripts in the same column are significant at $p< 0.05$.

Eva et al. (2006) noticed the DNA-protective effects of essential oils on hepatoma HepG2. They reduced the level of DNA damage induced by hydrogen peroxide (H_2O_2) associated with their antioxidant activity.

In the face of the fact that, plant volatile compounds appear to accumulate in the cell membrane and increase permeability, resulting in leakage of enzymes and metabolites ***(Tsai et al., 2007)***. In addition, they posses biological activities such as that of antioxidants ***(Miura et al., 2002)*** enhanced liver and kidney functions ***(Hermandez et al., 2004)***.

In this context, it is akin to mention that, aromatic plants and their essential oils can be used as antibacterial and hepato and renoprotective supplement in the developing countries towards the development of new therapeutic agents ***(Suvajdzic et al., 2006; Sylvestre et al. 2006)***.

Table (3): Effects of oral administration of Orego-stim®; 0.3ml / liter and Enrofloxacin(Opitryl)®;10 mg/kg B.W. of birds (1 ml / 2 liter) on serum total proteins, albumin, ALT and AST levels (IU/L) in chicks experimentally infected with *E. coli*.

Groups	Total Protein				Albumin				ALT				AST			
	Age in days				Age in days				Age in days				Age in days			
	15th	22nd	29th	36th	15th	22nd	29th	36th	15th	22nd	29th	36th	15th	22nd	29th	36th
1	2.64± 0.15^{C}	2.83± 0.14^{A}	2.33± 0.08^{A}	2.63± 0.08^{A}	1.42± 0.01^{A}	1.39± 0.03^{A}	1.45± 0.02^{A}	1.45± 0.02^{A}	27.3± 0.33^{D}	27.6± 1.45^{C}	26.6± 1.45^{A}	27.6± 0.33^{A}	67.3± 0.88^{D}	68.6± 1.85^{D}	64.0± 2.08^{A}	68.3± 1.20^{A}
2	3.82± 0.09^{A}	3.60± 0.17^{A}	2.43± 0.33^{A}	2.90± 0.23^{A}	1.00± 0.05^{B}	1.41± 0.01^{A}	1.42± 0.06^{A}	1.41± 0.02^{A}	41.6± 0.88^{A}	41.3± 1.85^{A}	28.0± 1.52^{A}	25.3± 0.66^{A}	86.3± 2.18^{A}	91.3± 1.85^{A}	64.3± 1.20^{A}	69.3± 1.45^{A}
3	3.00± 0.05^{B}	3.13± 0.18^{A}	2.40± 0.23^{A}	2.60± 0.11^{A}	1.38± 0.04^{A}	1.38± 0.04^{A}	1.42± 0.01^{A}	1.44± 0.02^{A}	35.6± 1.76^{B}	33.6± 1.85^{B}	25.6± 0.88^{A}	26.3± 0.88^{A}	76.6± 1.20^{B}	85.0± 0.57^{B}	65.0± 3.05^{A}	69.0± 4.93^{A}
4	1.93± 0.06^{D}	2.53± 0.29^{A}	2.23± 0.14^{A}	2.53± 0.14^{A}	1.40± 0.05^{A}	1.38± 0.03^{A}	1.43± 0.03^{A}	1.44± 0.03^{A}	32.0± 1.52^{C}	33.0± 0.57^{B}	25.3± 1.45^{A}	26.3± 1.45^{A}	73.6± 1.8^{BC}	79.6± 1.45^{C}	63.3± 2.84^{A}	70.0± 2.88^{A}
5	3.23± 0.08^{B}	2.96± 0.26^{A}	2.33± 0.17^{A}	2.40± 0.25^{A}	1.42± 0.01^{A}	1.40± 0.005^{A}	1.44± 0.03^{A}	1.43± 0.01^{A}	29.0± 0.5^{CD}	30.6± 0.3^{BC}	25.3± 2.40^{A}	28.0± 1.52^{A}	71.3± 1.8^{CD}	72.3± 1.45^{D}	64.6± 1.45^{A}	68.0± 2.30^{A}
6	3.27± 0.03^{B}	3.20± 0.35^{A}	2.40± 0.17^{A}	2.46± 0.26^{A}	1.42± 0.01^{A}	1.39± 0.02^{A}	1.44± 0.03^{A}	1.44± 0.02^{A}	28.6± 0.3^{CD}	31.0± 0.5^{BC}	27.0±2.08^{A}	28.3± 1.66^{A}	69.3± 0.3^{CD}	71.0± 1.15^{D}	65.3± 0.66^{A}	69.3± 4.17^{A}

Group (1) Non infected, non treated chicks (-ve control) Group (2) *E. coli* infected chicks (+ve control)

Group (3) Infected chicks & treated with the Orego-stim® Group (4) Infected chicks& treated with the therapeutic dose of enrofloxacin (Opitryl)®

Group (5) Infected chicks& treated with Orego-stim® and therapeutic dose of enrofloxacin (Opitryl)®

Group (6) Chicks given Orego-stim® from the 1st day old throughout the experimental period and infected with E. coli on the 5th day of age

Means carrying different superscripts in the same column are significant at $p< 0.05$

Table (4): Effects of oral administration of Orego-stim®; 0.3ml / liter and Enrofloxacin(Opitryl)®; 10 mg/kg of B.W. of birds1 ml / 2 liter) on serum uric acid and creatinine levels (mg/dl) in chicks experimentally infected with *E.coli*.

Groups	Uric A				Creatinine			
	Age in days				Age in days			
	15th	22nd	29th	36th	15th	22nd	29th	36th
1	8.63± 0.3^{CD}	8.26± 0.14^{C}	7.60± 0.30^{A}	7.50± 0.28^{A}	0.45± 0.01^{D}	0.48± 0.01^{D}	0.54± 0.02^{A}	0.53± 0.02^{A}
2	12.5± 0.81^{A}	12.6± 0.37^{A}	7.36± 0.32^{A}	7.53± 0.35^{A}	0.97± 0.06^{A}	0.90± 0.02^{A}	0.52± 0.03^{A}	0.54± 0.01^{A}
3	10.3± 0.6^{BC}	10.5± 0.28^{B}	7.60± 0.55^{A}	7.63± 0.40^{A}	0.68± 0.03^{C}	0.64± 0.03^{C}	0.52± 0.01^{A}	0.53± 0.02^{A}
4	11.5± 0.5^{AB}	11.2± 0.14^{B}	7.83± 0.57^{A}	7.73± 0.61^{A}	0.80± 0.02^{B}	0.71± 0.005^{B}	0.52± 0.04^{A}	0.53± 0.008^{A}
5	8.56± 0.3^{CD}	8.36± 0.18^{C}	7.46± 0.26^{A}	7.43± 0.40^{A}	0.52± 0.01^{D}	0.48± 0.01^{D}	0.55± 0.01^{A}	0.53± 0.02^{A}
6	8.26± 0.68^{D}	8.66± 0.44^{C}	7.50± 0.17^{A}	7.16± 0.38^{A}	0.48± 0.02^{D}	0.48± 0.01^{D}	0.53± 0.02^{A}	0.52± 0.03^{A}

Group (1) Non infected, non treated chicks (-ve control) Group (2) *E. coli* infected chicks (+ve control)

Group (3) Infected chicks & treated with the Orego-stim® Group (4) Infected chicks& treated with the therapeutic dose of enrofloxacin (Opitryl)®

Group (5) Infected chicks& treated with Orego-stim® and therapeutic dose of enrofloxacin(Opitryl)®

Group (6) Chicks given Orego-stim® from the 1st day old throughout the experimental period and infected with *E. coli* on the 5th day of age Means carrying different superscripts in the same column are significant at $p< 0.05$.

Conclusion

From the obtained results it could be concluded that, Orego-stim® can be considered a promising mixture of essential oils due to its high efficacy (growth performance, antibacterial and immunomodulating effects) and positive impact on both liver and kidney functions that may provide evidence for the hepato and renoprotective effects of the essential oils. The study highly recommend the use of Orego-stim® as a prophylactic agent in dealing with *E. coli* infection in chicken however, its concurrent administration with enrofloxacin in treatment of such case revealed the most favorable outcomes .

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Assessment of female adolescent Reproductive health needs in Assiut city

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Abstract: Adolescents around 17million in Egypt (2005) have different needs and require different counseling approaches and more information. Adolescence is a critical stage for risk-taking because adolescents are moving toward independence and tend to experiment and test limits**. Aim of this study**: was to determine the problems of female adolescents, and provide information about reproductive health. **Subject and methods**: Cross-sectional analytic study was conducted in nursing secondary schools and technical institutes in Assiut city in Upper Egypt with total number of 514 adolescent female students which are recruited. The data were collected through a self-administered questionnaire sheet and a physical assessment sheet. **Results**: The study revealed that 94.6% of the girls were circumcised, and 98.2% had their menarche. Overall, 65.5% had satisfactory knowledge, and 81.5% had positive attitude. The main sources of information were classroom whereas parents, newspapers, and magazines were less reported. There was a statistical significance difference between knowledge and had circumcision ($p=0.002$), also, between knowledge about reproduction and their related attitudes and beliefs ($p<0.001$). **Conclusion**: unmet information needs were related to female genital mutilation, family planning, pregnancy and labor, and the preferred sources are school book, media, and parents. **Recommendations:** It is recommended that the unmet needs identified should be used for development of educational programs for adolescents. The role of the parents, as well as health care providers needs to be fostered through using of multimedia as television and radio.

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Key words: Adolescents, reproductive health, knowledge, attitude.

1. Introduction:

Adolescents are inherently different from adults and therefore have different needs because they are less developed physically and psychologically; they have different cognitive abilities and skills, which requires different counseling approaches and takes more time; they tend to be less well informed and therefore require more information; conflicts between culture or parental expectations and the adolescents' emerging values present serious challenges for them; and they are in a transitional state and are not sure where they fit in ***(Carrera, 2006)***.

Adolescence is a critical stage for risk-taking because adolescents are moving toward independence and tend to experiment and test limits, including practicing risky behaviors; substance or drug use typically occurs for the first time during adolescence; sexual experiences (not always voluntary) usually begin during adolescence; consequences of risky behaviors can have serious and long-term effects; adolescents are experiencing pressures from peers, family, society, and the community; and adolescents are easily influenced ***(Eaton et al., 2010)***.

Adolescence is an opportunity time for professional interventions because health education can be integrated into school, home, and religious activities; life-long health habits are established during adolescence; interventions can help adolescents make good decisions and take responsibility for their actions, often preventing serious negative consequences in the future; and there are many effective channels for reaching adolescents such as, through schools, religious institutions, youth organizations, community and recreational activities, parental communication, peer education, the media, and health service facilities ***(Brener et al., 2006; Manlove et al., 2009)***.

Nearly half of the world population and 63 per cent in the least developed countries are currently below age 25, meaning that a large contingent of people will be entering reproductive life in the near future. The population classified as youth, between ages 15 and 24, is estimated to be 1 billion and it comprises nearly 18 per cent of the world population, approximately 14 percent of the population in the more developed regions and nearly 19 per cent of the population in the less developed regions. A large majority of the world's youth lives in less developed regions: 61 percent in Asia, in Egypt around17 million person at age of adolescent***(Brown and Suellentrop, 2009)***.

Very early, adolescents' reproductive rights have been defined as those rights that grant access to

reproductive health information and services, and rights that enable young people to decide freely and responsibly on all aspects of one's sexuality. This includes making informed decisions about sexual expression, relationships, and whether or when to have sex and whether or when to have children. These rights are or should be supported by policies that facilitate access to quality reproductive health information and services for all youth ***(Kelly and Schwartz, 2007)***.

The influence of the family environment on young people's onset of sexual activity is also increasingly acknowledged ***(Munthali et al., 2006)***. Parents and other family members usually play a central role in shaping youth's knowledge, values and attitudes, including those related to sexual and reproductive health. Several studies have documented the influential role of family stability, father's presence in the household and parent-teen communication on the timing of sexual initiation and the reductin of risk-taking behavior ***(Räsänen, 2009)***.

Aim of the study:

The aim of the present study was to determine the problems of female adolescents, and to provide information about reproductive health among them.

Subjects and Methods

Research design& Setting

A cross-sectional analytic research design was used in carrying out the study. It was conducted in nursing secondary schools and technical institutes in Assiut city in Upper Egypt.

Subjectsand Sample size:

The subjects of this study consisted of 514 female adolescents in nursing secondary schools and technical institutes in Assiut city.

The sample size was estimated to determine an expected satisfactory knowledge rate among adolescent girls of 50% or more, with 5% absolute precision and 95% level of confidence. Using the single proportion equation for dichotomous variables:

$$n = \frac{(z_{\alpha/2})^2 p(1-p)}{D^2}$$

Where:

n = sample size

p = 0.50

D = 0.50 x10% = 0.05

The estimated sample size is 384 subjects. After adjustment for a dropout rate of 25%, the sample size was increased to 514.

Fieldwork

Data collection was carried out over a period of four months, from February 2007 to May 2007. After obtaining official approvals, the researcher visited the study settings and met with the headmasters to explain the purpose and procedures of the study. This was followed by recruitment of students from various school grades. The researcher met with them and explained the purpose of the study, the components of the tool, and how to answer the questions. Filling the questionnaire took from 25 to 30 minutes for each student. The researcher was present to answer any questions.

Data collection tool

The data of this study were collected using a self-administered questionnaire sheet and a physical assessment sheet.

Self-administered questionnaire developed by the researcher based on review of pertinent data. The tool was reviewed by nursing and medical experts in obstetrics and gynecology reproductive health, as well as community health. The finalized tool included the following parts:

1st part: Socio-demographic 2nd part : history of circumcision, age, and associated complications, 3rd part: Menstrual history, age at menarche, and 4th part: Knowledge about reproductive health. After completion of the questionnaire, each student was examined using the physical assessment form. Weight and height and pelvic girth were measured according to standard methods ***(Larsson et al., 1984, Himes and Dietz, 1994)***. This was followed by assessment of the vital signs. A special room was prepared in the school for physical assessment by the researcher in special room in order to ensure privacy.

Scoring:

For the knowledge items, a correct response was scored 1 and the incorrect zero. For each area of knowledge, the scores of the items were summed-up and the total divided by the number of the items, giving a mean score for the part. These scores were converted into a percent score. Knowledge was considered satisfactory if the percent score was 60% or more and unsatisfactory if less than 60%.

Physical assessment sheet: developed by the researcher to record the physical examination findings. These included:

Anthropometric measurements: weight and height to calculate to body mass index (BMI) as kg/m^2 height, and pelvic girth by tape measure

Vital signs: pulse, respiration, blood pressure.

Pilot study

After development and validation of the tools, a pilot study was conducted on 50 adolescent girls representing about 10% of the sample size. Its purpose was to test the feasibility of the tools, and to identify any problems related to sequence and clarity of the questions. It also helped the researcher to estimate the time needed to fill the forms. The pilot study findings showed that the questions were clear and relevant, but some wording was modified to increase clarity. The tools were finalized and made ready for use based on the pilot study findings.

Ethical considerations

All principles of ethics in research were followed in carrying out the study. The study protocol was approved by pertinent committees. The purpose of the study was explained to participants, and their written consent to participate in the study was obtained. They were informed about their right to refuse participation, or to withdraw at any time with no reason to be given. They were assured that the information would be totally confidential and used for research purpose only. The questionnaire forms were anonymous. Professional help was provided in case of need. if any of them had a problem referral are done.

Limitations of the study

Some students were reluctant to participate for fear that the questionnaire would affect their academic evaluation. They were convinced about confidentiality, and the forms were anonymous. Others were embarrassed of having the physical assessment, but securing a private place for examination solved the problem.

IV. Statistical Design

Data entry and statistical analysis were done using SPSS 14.0 statistical software package. Data were presented using descriptive statistics in the form of frequencies and percentages for qualitative variables, and means and standard deviations for quantitative variables. Qualitative categorical variables were compared using chi-square test. Whenever the expected values in one or more of the cells in a 2x2 tables was less than 5, Fisher exact test was used instead. Statistical significance was considered at p-value <0.05.

3. Results:

Table (1): describes the socio-demographic characteristics of adolescent girls in the study sample. Their mean age was 16.2±1.1 years, and the highest percentage was in the age category 16 to <17 years (34.0%). Slightly more than half of them (54.3%) had urban residence. The highest percentage was in first and second secondary years (37.9% and 36.2%, respectively). The majority mentioned that the curriculum covered information about reproduction (92.2%). Regarding parents' socio-demographic characteristics shows that about one-fifth of the fathers (20.6%) and slightly less than half of the mothers (47.7%) were illiterate. Meanwhile, only 5.8% of the fathers and 1.8% of the mothers had university education. About two-thirds of the fathers were manual workers (64.2%), whereas the majority of the mothers were housewives (88.7%).

Table (2): demonstrates that the majority of the studied girls were circumcised (94.6%). The age at circumcision varied between less than one to 17 years, with a mean 8.8±2.7 years. He majority of the circumcised girls reported having had related acute problems (86.0%).and done by physician at clinic

Table (3): shows that almost all studied girls had their menarche (98.2%). The cycle was mostly regular (65.5%), with duration of 28 to 30 days (70.7%), three to four days menstrual period (72.5%), and an average number of pads (78.0%). The majority knew that the age at menarche ranged between 12 and less than 16 years (89.1%). As regards the beliefs related to things to be avoided during menses, the majority mentioned prayer (92.2%), followed by Ramadan fast (72.8%) and sexual intercourse (53.7%). Meanwhile, the majority (94.7%) were bathing during menses. Only less than half of the sample had correct knowledge about the time of ovulation (48.4%). In total, 42.6% of the girls had correct knowledge about menstruation, and slightly less than half of them (49.8%) had related positive attitude.

Figure (1): describes the prevalence of menstrual pain in the study sample. It indicates that more than half of the girls had moderate pain (54.5%).

Figure (2): demonstrates that school book was the main source (85.6%), followed by educational media (15.6%). Only 12.8% mentioned parents as sources of information.

Table (4): illustrates the anthropometric measurements of the studied girls. Concerning BMI, it indicates that the majority (70.2%) were normal. Meanwhile, 11.7% and 3.5% were respectively overweight and obese. Meanwhile, slightly less than half of the sample had their girth 90 to less than 100 cm, with a mean 96.0±12.1 cm.

Table (5): illustrated that the majority reported having seen a related diagram (97.9%), mostly in anatomy books (96.2%). Slightly less than half of the sample (45.9%) has correctly mentioned five female genital organs. However, 3.3% of the girls could not name any organ. The table also shows that the highest

percentage of the girls could correctly mention only one part of the vulva (38.5%), and 23.0% correctly mentioned its four parts. The majority had correct knowledge about the location of the female genitalia (90.1%), and the site of implantation (96.1%). Conversely, only one-third correctly knew the source of the menstrual blood (33.3%), and about two-fifth correctly knew the site of sexual intercourse (41.8%). Overall, slightly less than half of the girls had satisfactory knowledge (48.1%).

Table (6): showed that the majority had positive attitude towards age for marriage (90.3%), and pregnancy spacing (79.8%). For the majority of girls, breastfeeding should be two years (89.1%). Overall, slightly more than three-fourth of them had satisfactory knowledge about marriage (75.3%), and 58.0% had related positive attitudes and beliefs.

Table (7): illustrated that the majority knew that diseases could be transmitted by sexual relations (99.4%). The main complications mentioned were infertility (51.0%), followed by urinary tract infection (48.1%), and vaginal secretions (44.2%). The table also shows that the majority (98.8%) knew about AIDS, its manifestations (74.5%), lack of treatment (87.7%), and possible mother-fetal transmission (97.5%). Also, almost all studied girls agreed upon the importance of premarital counseling (98.4%). In total, slightly more than half of the girls (54.1%) had satisfactory knowledge about STDs, and the majority (98.4%) had related positive attitude.

Table (8): showed the majority had correct knowledge about contraceptive pills (96.1%), IUD (92.8%), and other family planning methods (90.7%). Conversely, only 14.4% of them had correct knowledge about the effectiveness of IUD. In total, about three-fourth of the girls had satisfactory knowledge about family planning (74.1%).

Information needs and preferred sources

Table (9): Concerning studied girls' information needs, Table 19 shows that slightly more than half of them (54.7%) have expressed such need. The needed information was mostly for FGM (38.4%), followed by family planning (7.8%), and pregnancy and labor (6.4%). The lowest needs were related to male genital system (0.7%), and male contraception (0.4%). The preferred sources of information were mainly school book (75.5%), educational media (21.2%), and parents (14.4%).

4. Discussion

The adolescence is a period of preparation for adulthood. During this time several key developmental tasks are undertaken. These include physical and sexual maturation, movement toward social and economic independence, and the development of identity. Behavior patterns established during this process, such as drug use or non-use and sexual risk taking or protection can have a long-lasting positive or negative impact on future health and wellbeing. As a result, it is during this process that providers can have the greatest impact on helping young people establish healthy behaviors (Coyle *et al.,* 2003).

The study was carried out on a sample of adolescent girls with age ranging between 14 and 20 years. This age range covers the middle and late adolescence stages as defined by the NCERT (1999). Accordingly, the mid adolescence stage is between 14 and 15 years, and is characterized by the development of a separate identity from family, and tendency to more relationships with peer groups and other sex. The stage of late adolescence is between 16 and 19 years, and is characterized by full physical development, and clear separate identity. Therefore, these stages are more suitable for assessment of issues related to reproductive health.

Slightly more than half of the girls in the present study were from urban areas. This provides a good mix of a sample representing both urban and rural communities, which have different perceptions regarding reproductive health issues. They were selected from nursing schools and institutes for two main reasons. Firstly, they must have better knowledge about reproductive health issues compared to girls in other educational sectors. In support of this, the great majority of them confirmed that their school curricula covered information regarding reproduction. Secondly, their future career implies that they themselves will have the role of educating adolescents regarding health in general, and reproductive health in particular. Therefore, it was deemed important to assess their related knowledge and beliefs.

The description of the socio-demographic characteristics of the parents of the present study adolescent girls is of great importance in shaping their knowledge and beliefs regarding reproductive health. It was found that one-fifth of the fathers about half of the mothers were illiterate. Also, most of the fathers were manual workers, while the majority of the mothers were housewives. These characteristics indicate a low socio-economic level, which will have its repercussions on the study findings as will be shown.

Table 1. Socio-demographic and educational characteristics of adolescent girls and their parents in the study sample (n=514)

Socio-demographic and educational characteristics	Frequency	Percent
Age (years):		
<16	161	31.3
16-	175	34.0
17-	123	23.9
18+	55	10.7
Range	14-20	
mean±SD	16.2±1.1	
Residence:		
Urban	279	54.3
Rural	235	45.7
Nursing education level:		
1st secondary	195	37.9
2nd secondary	186	36.2
3rd secondary	114	22.2
Technical institute	19	3.7
Number of years of education:		
Range	9-14	
mean±SD	9.9±1.0	
Curriculum covers information about reproduction:		
No	40	7.8
Yes	474	92.2
Father education:		
Illiterate	106	20.6
Read/write & Primary	198	38.5
Preparatory& Secondary	190	35
University	30	5.8
Father job:		
Employee	130	25.3
Manual worker	330	64.2
Unemployed	54	10.5
Mother job:		
Housewife	456	88.7
Working	58	11.3
Mother education:		
Illiterate	245	47.7
Read/write & Primary	168	32.3
Preparatory& Secondary	94	18.3
University	9	1.8

Table 2. History of circumcision (FGM) among adolescent girls in the study sample (n=514).

	Frequency	Percent
Had circumcision:		
No	28	5.4
Yes	486	94.6
Age at circumcision in years (n=486):		
<5	28	5.8
5-	233	47.9
10+	225	46.3
Range	<1-17	
mean±SD	8.8±2.7	
Median	9	
Had acute problems (n=486)	418	86.0
Who did the circumcision	458	89.2
By physician or nurse		5.4
By daya	28	

Table 3. Menstrual history and knowledge, attitude & beliefs about menstruation among adolescent girls n the study sample (n=514)

Menstrual history and knowledge, attitude & beliefs about menstruation	Frequency	Percent
Had menarche:		
No	9	1.8
Yes	505	98.2
Age at menarche (years):		
<12	50	9.7
12-	458	89.1
16+	6	1.2
Regularity:		
Regular	331	65.5
Irregular	174	34.5
Things to be avoided during menses:		
Prayer	474	92.2
Ramadan fast	374	72.8
Sexual intercourse	276	53.7
Physical exercises	48	9.3
Going to church	46	8.9
Bathing during menstruation:	487	94.7
Correct time of ovulation	249	48.4
Knowledge about menstruation:		
Satisfactory (60%+)	219	42.6
Unsatisfactory (<60%)	295	57.4
Beliefs and attitudes related to menstruation:		
Positive (60%+)	256	49.8
Negative (<60%)	258	50.2

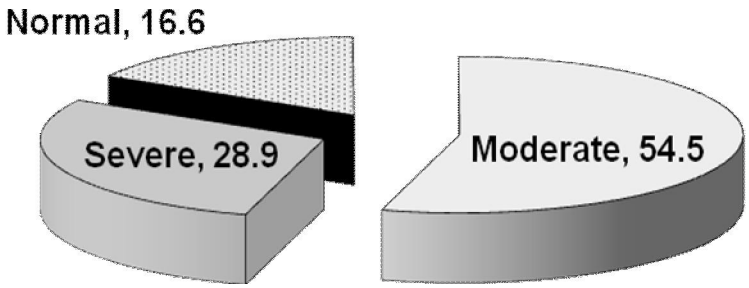

Figure 1. Prevalence of menstrual pain in the study sample (n=514).

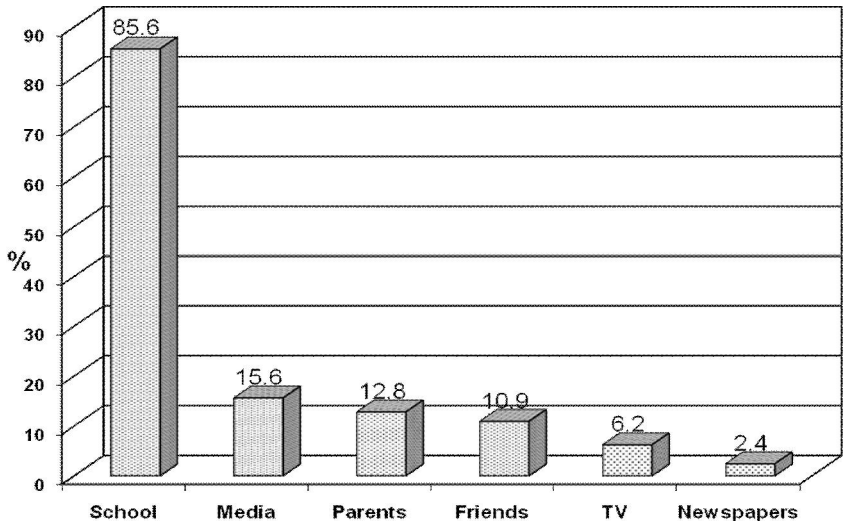

Figure 2. Sources of information about menstruation among adolescent girls in the study sample (n=514)

Table 4. Anthropometric measurements of adolescent girls in the study sample (n=514)

Anthropometric measurements	Frequency	Percent
BMI:		
<18.5 (underweight)	75	14.6
18.5- (normal)	361	70.2
25- (overweight)	60	11.7
30+ (obese)	18	3.5
Range	14.0-55.0	
mean±SD	22.1±4.5	
Girth (cm):		
<90	87	16.9
90-	242	47.1
100+	185	36.0
Range	34.0-151.0	
mean±SD	96.0±12.1	

Table 5. Knowledge about anatomy and physiology of female genital system among adolescent girls in the study sample (n=514)

Knowledge about anatomy and physiology of female genital system	Frequency	Percent
Had seen a diagram of female reproductive system:		
Saw diagram in (n=503):		
Anatomy book	484	96.2
School poster	2	0.4
Hospital poster	17	3.4
Correctly know:		
Location of female genitalia	463	90.1
Source of menstrual flow	171	33.3
Site of sexual intercourse	215	41.8
Site of ovum ready for fertilization	250	48.6
Number of fertilizable ova produced monthly	263	51.2
Site of fertilization (ovum and sperm)	262	51.0
Site of implantation	494	96.1
Total knowledge about anatomy/physiology of female system:		
Satisfactory (60%+)	247	48.1
Unsatisfactory (<60%)	267	51.9

Table 6. Knowledge, attitude, and beliefs about marriage and pregnancy among adolescent girls in the study sample (n=514)

Knowledge, attitude, and beliefs about marriage and pregnancy	Frequency	Percent
Appropriate age for marriage (years):		
Range	15-30	
mean±SD	20.3±1.9	
Median	20	
Positive attitude/ correct belief towards:		
Age for marriage	464	90.3
Pain during sexual intercourse	351	68.3
Bleeding in first marital sexual intercourse	380	73.9
Pregnancy spacing	410	79.8
Pain during labor	183	35.6
Duration of breastfeeding (years):		
<2	31	6.0
2	458	89.1
>2	25	4.9
total	458	89.1
Total knowledge about marriage and pregnancy:		
Satisfactory (60%+)	387	75.3
Unsatisfactory (<60%)	127	24.7
Beliefs and attitudes related to marriage and pregnancy:		
Positive (60%+)	298	58.0
Negative (<60%)	216	42.0

Table 7. Knowledge, attitude, and beliefs about sexually transmitted diseases (STDs) among adolescent girls in the study sample (n=514)

Knowledge/attitude/beliefs about sexually transmitted diseases (STDs):	Frequency	Percent
Correctly know:		
Diseases can be transmitted by sexual relations	511	99.4
Complications of STDs:		
Infertility	262	51.0
Urinary tract infection	247	48.1
Vaginal secretions	227	44.2
Death	180	35.0
Urinary secretions	119	23.2
Heart diseases	45	8.8
AIDS	508	98.8
Manifestations of AIDS	383	74.5
Lack of treatment for AIDS	451	87.7
Modes of transmission of AIDS:		
Sexual intercourse	483	94.0
Blood transfusion	392	76.3
Shared syringes	368	71.6
Transmission from mother to fetus	501	97.5
High-risk groups	316	61.5
Importance of pre-marital counseling	506	98.4
Total knowledge about STDs:		
Satisfactory (60%+)	278	54.1
Unsatisfactory (<60%)	236	45.9
Beliefs and attitudes related to STDs:		
Positive (60%+)	506	98.4
Negative (<60%)	8	1.6

Table 8. Knowledge about family planning among adolescent girls in the study sample (n=514)

Knowledge about family planning:	Frequency	Percent
Know about contraceptive pills (OCP)	494	96.1
Know the time of use of OCP	382	74.3
Know about IUD	477	92.8
Know duration of effectiveness of IUD	74	14.4
Site if IUD insertion	407	79.2
Local methods used by men	355	69.1
Other family planning methods	466	90.7
Permanent family planning methods for woman	377	73.3
Permanent family planning methods for men	241	46.9
Knowledge about family planning:		
Satisfactory (60%+)	381	74.1
Unsatisfactory (<60%)	133	25.9

Table 9. Information needs and preferred sources of information about reproductive health as reported by adolescent girls in the study sample (n=514)

	Frequency	Percent
Need more information about reproductive health:		
No	233	45.3
Yes	281	54.7
Information needed (n=281):[@]		
FGM	108	38.4
Family planning	22	7.8
Pregnancy and labor	18	6.4
Menstruation	17	6.0
Adolescence	13	4.6
Sexual intercourse	13	4.6
AIDS	13	4.6
Related religious issues	5	1.8
Premarital counseling	5	1.8
General information	3	1.1
Early marriage	3	1.1
Female genital system	3	1.1
Infertility	3	1.1
Personal hygiene during menses	2	0.7
Male genital system	2	0.7
Male contraception	1	0.4
Preferred sources of information:		
School book	388	75.5
Educational media	109	21.2
Parents	74	14.4
TV	65	12.6
Friends	33	6.4
Magazines	22	4.3
Newspapers	19	3.7

(@) Not mutually exclusive

The very high prevalence of circumcision in the present study is expected given the low socio-economic conditions of their parents, with high illiteracy. Also, about half of them were from rural areas. In line with this, the study of Elnashar and Abdelhady (2007) revealed that 75.8% of the studied sample was circumcised. This unexpected low rate reflects the trend towards rejection of circumcision particularly in some classes. It was attributed to the higher educational levels of the participants, where

no illiterates were found among the non-circumcised females and the majority of them were at university level or higher; additionally, the majority was working and living in urban residential areas.

According to the present study findings, the majority of the circumcised girls reported having had related acute problems. This high rate of post-circumcision complications is in line with previous studies that reported serious complications, tragic deaths, and extensive reproductive health problems among young women following this procedure (Larsen and Okonofua, 2002; Yoong *et al.,* 2004; Braddy and Files, 2007).

Almost all adolescent girls in the present study had their menarche. This is quite plausible since the youngest age in the sample was 14 years old, which is beyond the usual age at menarche. Similar age at menarche was previously reported. For example, the average statistically weighted worldwide age at menarche was found to be 13.54 years (Garg *et al.,* 2001). Meanwhile, El-Gilany *et al.* (2005) in Egypt found that the age at menarche ranged from 10-16 years, with a mean and median of 12.9 and 13 years, respectively. This median is much higher compared to the present study, and this might be explained by differences in study settings.

According to adolescent girls' reporting, about one third had irregular cycles, with longer or shorter duration of the cycle, and longer bleeding days, and more than half of them had menstrual pain, mostly of moderate severity. Also, about one-third of them had more or less than average number of pads. This average, as reported by El-Gilany *et al.* (2005) was 2.5 times per day. Concerning the irregularities in time and bleeding, this might be due to that menstruation was at its start among the girls in the study sample, and this is usually associated with irregularities due to hormonal imbalance. However, Elnashar and Abdelhady (2007) had another explanation. They found that circumcised women had more irregularity of the cycle and more dysmenorrheal, which can result from chronic pelvic infection and is also caused by pelvic congestion. Menstrual flow may be retained due to tiny vaginal opening. Scarring can be the cause of genital infection and disturbances of menstruation.

Concerning physical findings, the majority of the adolescent girls in the present study had normal body mass index (BMI). Meanwhile, about 15% were overweight or obese, and an almost similar percentage was underweight. These findings indicate that obesity is not highly prevalent among them, but on the contrary, they have a problem with underweight. The finding might be explained by the low socio-economic level of their families. In fact, the socioeconomic status has been previously reported to be associated with underweight and obesity (Rennie and Jebb, 2005).

In congruence with the percentages of overweight found in the present study, Gordon-Larsen *et al.* (2004) showed similar levels of incident overweight in the transition from adolescence to young adulthood. The 5-year incidence of overweight in participants in their study ranged from 11.2% in the age category 13-15 years, to 14.6% in those who were 18-20.

However, in disagreement with the present study findings, a study of 9-16-year old rural youth demonstrated higher rates, where 15% of adolescents were chronically obese (Mustillo *et al.,* 2003). The high rate of obesity was attributed to the rapid growth rates that make adolescents vulnerable to excess weight gain, in addition to a variety of social factors that contribute to adolescent obesity including eating and physical activity patterns, especially TV viewing and other types of sedentary physical activity. Therefore, the importance of adolescence as a critical period for development of overweight may depend on the prevalence of many other obesigenic risk factors (Wardle *et al.,* 2006).

On the other hand, lower rates of overweight and obesity, compared to the present study were reported elsewhere. Thus, Vissers *et al.* (2008) demonstrated that in a sample of 16-18-year-old adolescents in Belgium, 9.9% were overweight and 3% were obese. Studies in other European countries also showed low prevalence of overweight and obesity among adolescents (International Association for the Study of Obesity, 2004; Janssen *et al.,* 2005).

As for the sources of information about female and male genital system anatomy and physiology, the great majority of the present study adolescent girls reported that the main source was school. In agreement with this finding, *de Irala et al. (2008)* highlighted that middle and high schools' textbooks are the most common educational resource, making them the backbone of the official curriculum and, to a greater extent, of the culture transmitted in the classroom. However, in Spanish schools, sexual education is a subject that can be covered by any teacher. Sometimes, specific courses about sexuality are given by a school counselor and aimed at parents and/or pupils. However, most education about sexuality is given in biology lessons.

However, in disagreement with the present study results, *Ancheta et al. (2005)* mentioned that most studied adolescent girls reported receiving reproductive health education from both parental (80%) and formal sources (92%). Parents discussed the menstrual cycle more frequently than other sex education topics, while formal sources focused most on teaching about sexually transmitted disease

(STDs). The authors also reported that early reproductive health education and education from both parental and formal sources is associated with reduced sexual risk among high-risk adolescent girls.

Concerning knowledge, attitude, and beliefs related to menstruation, the present study revealed that almost a half of the adolescent girls had negative attitudes and related misconceptions. Their knowledge about ovulation and sexual intercourse during menstruation was low. On the other hand, the majority knew the age at menarche, avoided prayer and Ramadan fast, and bathed during menses. Their correct knowledge about age at menarche and religious practices are expected since this is part of their experience and practices. On the other hand, the low knowledge about sexual intercourse during menses is attributed to the conservatism and restrictions on discussing these issues. In agreement with these study findings, *El-Gilany et al. (2005)* reported that the majority of the studied girls were taking showers during menstruation. Also, girls in a study in India had comp8arable findings, and they expressed their need for more information *(Garg et al., 2001)*.

Similar to knowledge about the genital system anatomy and physiology, the main source of information about menstruation among adolescent girls in the present study was the school book. The role of the media and parents was minimal in provision of information. This might be attributed to that according to cultural and traditional beliefs in Egypt menstruation is not considered an appropriate topic of discussion, which leads to the lack of accurate and available information among adolescents. Also, in school most young adolescents are probably too embarrassed to ask questions. Moreover, they might not even have a chance to ask questions.

These present study findings the majority of girls the number of pads changed are available(4-6)pads78%also reported the main source of information about menstruation are school book(85.6)are in disagreement with *El-Gilany et al. (2005)* who found that almost all the girls reported the mass media as their source of knowledge about menstrual hygiene, followed by mothers. In contrast, an earlier study in Alexandria demonstrated that mothers were the most common source of information on the physiology of menstruation and care before and after menarche, with school curricula and the media having a limited role *(El-Shazly et al., 1990)*.

The majority of the present study adolescent girls had positive attitude towards age for marriage, pregnancy spacing, and breastfeeding to be two years. In total, more than three-fourth of them had satisfactory knowledge about marriage, and more than half had related positive attitudes and beliefs. The positive attitude towards pregnancy spacing is quite important since closely spaced pregnancies among teenagers will, by definition, increase the risk of repeat teen pregnancies *(Gold et al., 2004)*. The optimal interpregnancy interval for preventing adverse birth outcomes appeared to be approximately 18-23 months *(Zhu et al., 1999; 2001; Zhu and Le, 2003)*

Regarding present study adolescent girls' knowledge, attitude, and beliefs related to sexually transmitted diseases (STDs), the findings point to high general knowledge, especially as related to AIDS, its manifestations, lack of treatment, and possible mother-fetal transmission. This high knowledge about AIDS might be explained by the fact that the mass media give great importance to this disease because of its seriousness, but disregard other more common STDs as gonorrhea and herpes. In congruence with this, it was found that a considerable number of adolescents in the U.S. have never heard of chlamydia or gonorrhea, whereas many adolescents have heard of AIDS *(Clark et al., 2002; Zamora et al., 2006)*.

Meanwhile, none of their socio-demographic characteristics was significantly related to their knowledge, attitude and beliefs related to reproduction. The only exception was association between satisfactory knowledge and having had circumcision. This might be explained by the experience the circumcised went through during the process of FGM, which might have increased their knowledge about reproductive system.

Furthermore, the present study revealed a statistically significant association between adolescent girls' knowledge about reproduction and their related attitudes and beliefs. It was noticed that more girls with satisfactory knowledge had positive attitudes. The finding indicates the influence of knowledge on attitudes and beliefs, and this should lead to fostering knowledge in order to correct negative attitudes and false beliefs.

The unmet information needs about reproductive health among the present study adolescent girls were mostly related to FGM, family planning, and pregnancy and labor. Similar unmet needs were reported in the study carried out by *Bansah et al. (2007)*. They also indicated that prenatal classes needed to be flexible so that if any new issue arose, it could be discussed. Moreover, *Gagnon (2001)* showed that participants wanted to receive specific information that was not necessarily what health-care providers taught, and this was probably due to their cultural values.

Conclusion

The study findings lead to the conclusion that the great majority adolescent nursing students, as other female adolescents in Egypt are circumcised, and had related acute problems. Overweight and obesity are not major problems among them. Only less than two-thirds of them have satisfactory knowledge about reproductive health, although the majority have positive attitude. Knowledge is significantly related to having had circumcision, and also to attitudes and beliefs. Their main sources of information are classroom while parents, health care providers, and media are less reported. The preferred sources of information are school book, educational media, and parents. If this are the findings among nursing students who ought to know better about health-related issues, worse is expected among other adolescents who had no chance to study them.

Recommendations

Based on the study findings, the following recommendations are suggested.

The unmet information needs identified in the study should be the base for development of educational programs for adolescents regarding reproductive health.

Since school was mentioned as the most preferred source of information, this raises the issue of including reproductive health topics in their curricula, not only nursing schools, but also general schools.

The false beliefs identified in relation to menstruation, female genital mutilation, marriage and pregnancy, and home responsibilities must be addressed in health education programs designed for adolescents.

The principles of adult learning with open discussions and active participation of attendants must be followed in any training programs for reproductive health for adolescents.

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Ameliorative Effect of Aqueous Leave Extract of *Ocimum Basilicum* on Ccl_4 - Induced Hepatotoxicity and Apoptosis in Albino Rats

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Abstract: Chemical-induced liver injury depends mostly on the oxidative stress. Basil or sweet basil (*Ocimum basilicum*) is known to have numerous pharmacological activities. The present study **aims** to investigate the effect of basil on Ccl_4-induced hepatotoxicity and apoptotic in albino rats. The result showed CCl_4 caused impairment of the normal structural organization of the hepatic lobules, congestion and dilatation of blood vessels, cytoplasmic vacuolization of the hepatocytes, leucocytic infiltrations and fatty degeneration. The biochemical results showed that there was an increase in serum level of ALT , AST, ALP, cholesterol , triglyceride, , LDL and HDL. Moreover, CCl_4 induced hepatic apoptosis. Treating animals with CCl_4 and aqueous leaves extract of *O. basilica* led to an improvement, in both histopathological and biochemical alterations induced by CCl_4. Also, apoptosis was repaired by shared administration with both *O. basilicum* and CCl_4. These results proved that *O. basilica* had an ameliorative effect against liver injury produced by **CCl_4** due to its antioxidant activity.
[Saber A. Sakr, Sabah F. El-Abd, Mohamed Osman, Asmaa M. Kandil, Mona S. Helmy **Ameliorative Effect of Aqueous Leave Extract of *Ocimum Basilicum* on Ccl_4 - Induced Hepatotoxicity and Apoptosis in Albino Rats**. Journal of American Science 2011; 7(8): 116-127]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: CCl_4 – Hepatotoxicity- rat- *O. basilicum* - Apoptosis

1. Introduction:

Oxidative stress has been shown to play a very crucial role in some diseases including liver disease. Free radical that generate inside the body is responsible for oxidative stress and compounds that can scavenge free radicals have great potential in ameliorating these disease processes (**Sies, 1997**). Basil or sweet basil (*Ocimum basilicum)* is a plant that belongs to the family Labiatae and is known as Tulsi in Hindi , Holy Basil in English and Rehan in Egypt. It is known to have numerous pharmacological activities. Many studies have established that basil leaves extracts have potent antioxidant , anti-aging , anticancer , antiviral , and antimicrobial properties (**Chiang *et al.*, 2005 ; Bozin *et al.*, 2006 ; Manosroi *et al.*, 2006 ; Almeida *et al.*, 2007, Akujobi *et al.*, 2010**). **Orafidiya *et al.* (2006)** investigated the efficacy of the leaf essential oil of *Ocimum gratissimum* Linn. in promoting hair growth in cyclophosphamide-induced hair loss. The results showed that ocimum oil may be capable of enhancing normal hair growth and promoting follicular proliferation in cyclophosphamide-induced hair loss. **Sethi (2003)** reported that leaves of *ocimum sanctum* possess good antioxidant as well as antistress potentials in experimental animals. Consumption of basil or basil oil has been associated with a reduction in total cholesterol, low-density lipoprotein and triglyceride (**Hicham *et al.*, 2009**). **Batra and Gupta (2006)** indicated that *Ocimum sanctum* leaf supplementation reduced the severity of hydropericardium, hepatitis, myocarditis accompanied with haemorrhages, oedema in lungs, lymphocytic depletion in lymphoid organs and focal interstitial nephritis. **Rupert (2009)** reported that basil or basil oil have agents for prevention and treatment of cardiovascular disease. It has also been shown that OS leaf extracts can protect the liver from heavy metals (**Sharma *et al.*, 2002**) and prevent isoproterenal induced myocardial necrosis in rats (**Sood *et al.*, 2005**).

Chemical-induced liver injury depends mostly on the oxidative stress in hepatic tissue. Carbon tetrachloride (CCl4)-induced liver damage is the best characterized system of xenobiotic-induced hepatotoxicity and is a commonly screening model to evaluate the hepatoprotective potential of drugs with antioxidant properties. Administration of CCl_4 causes extensive changes in liver morphology including steatosis, inflammation and necrosis (**Qiu *et al.*, 2005**). It induced liver fibrosis, cirrhosis, enhanced lipid peroxidation, increases ALT and causes collagen deposition in liver tissue (**Nan *et al.*, 2002, Campo *et al.*, 2004**). **SuYanga *et al.* (2008)** reported that single oral dose of CCl_4 produced significantly elevated levels of serum ALT, AST activities and extensive liver necrosis and fatty changes .Carbon tetrachloride was metabolized in

the liver by cytochrome P450 of the endoplasmic reticulum with the formation of a highly toxic trichlorometheyl radical (CCl_3) (**Conner *et al.*, 1990**).

2. Material and Methods

Animals

Male albino Wistar rats weighing 100 ± 5 g were kept in the laboratory under constant conditions of temperature (24 ± 2 ^{o}C) for at least one week before and through the experimental work, being maintained on a standard diet composed of composed of 20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitaminzed starch. Water was available *ad-libitum*.

Preparation of ocimum extract

Fresh leaves of *Ocimum basilicum* were collected from a garden within Genetic Engineering and Biotechnology Research Institute, Menoufia University, Sadat City, Egypt.The leaves were rinsed with clean water to remove any foreign matter. Leaves were blended with distilled water. The mixture was strained, the merc pressed and the mixture was filtrated using filter paper. The aqueous extract was used at a dose level of 20 ml/kg O. *basilicum* (**Offiah and Chikwendu,1999**).

Experimental design

All the experiments were done in compliance with the guide for the care and use of laboratory animals. Animals were divided into four groups:

Group1. Animals were fed on the standard diet and were served as control group.

Group2. Animals of this group were administrated with oral aqueous O. *basilicum* extract at a dose level of 20 ml/kg twice a week for 6 weeks.

Group3. Rats were injected intraperitonealy with 1.0ml/kg b.w of 10% CCl_4 dissolved in olive oil twice a week for 6 weeks (Sakr et al.2010).

Group4. Rats were injected with CCl_4 (1.0 ml/kg) followed by oral administration with aqueous O. *basilicum* extract at a dose level of 20ml/kg twice a week for 6 weeks.

Histological examination

The treated animals and their controls were sacrificed by decapitation after 2, 4 and 6 weeks of treatment. Liver was removed and fixed in Bouin's fluid. Fixed materials were embedded in paraffin wax and sections of 5 micrmetres thickness were cut. Slides were stained with haematoxylin and eosin for histological examination.

Biochemical assays

For biochemical assays blood was collected and centrifuged at 3000 rpm for 10 minutes and stored at -20 ^{0}C .Liver function enzymes ALT and AST were determined in serum according to the method of **Gella *et al.* (1985)**.The activity of alkaline phosphatase was assayed by the method of **El-Aaser and El-Marzabani (1975)**. Cholesterol and triglycerides were measured using the methods of **Zlaktis *et al.* (1953),** and **Fassati and Prencipe (1982)**, respectively.

DNA Fragmentation Assay

As a measure of apoptotic DNA fragmentation, the presence of DNA ladder was determined according to **Wlodek *et al.* (1991).** Extraction of DNA was done according to method of Hassab El- Nabi (2009) and Aljanabi S. M. (1997), 10 mg of liver tissue in eppendorf tubes were lysed with 600 microlitre buffer (50 mM NaCl, 1 mM Na_2EDTA, 0.5% SDS, PH 8.3) and gently shaked. The mixure was incubated overnight at 37 C^{O} then, 20 microlitre of saturated NaCl was added the sample, shacked and centrifuged at 12,000 rpm for 10 min. the supernatant was transferred to new Eppendorf tubes and then DNA precipitated by 600 microlitre cold isoproprnol. The mix was inverted several times till fine fibers appear, and then centrifuged for 5 min. at 12,000 rpm. The supernatant is removed and the pellets were washed with 500 microlitre 70% ethyl alcohol centrifuged at 12,000 rpm for 5 min. After centrifugation the alcohol was decanted or tipped out and the tubes plotted on Whatman paper to be dry. The pellets were resuspended in 50 microlitre or appropriate volume of TE buffer (10 mM Tris, 1 mM EDTA, PH 8). The resuspended DNA was incubated for 30 - 60 min with loading mix (Rnase + loading buffer) and then loaded into the gel wells.

Agarose gel electrophoresis

A gel was prepared with 2% agarose containing 0.1% ethidium bromide (200 ug/ml). The DNA samples were mixed with loading buffer (0.25% bromophenol blue, 0.25% xylene cyanole FF and 30% glycerol) and loaded into the wells (2 ug of DNA/lane) with a standard molecular- sized ladder marker (Pharmacia Biotech., USA). The gel was electrophoresed at a current of 50 mA for 2.5 h using the submarine gel electrophoresis machine. The DNA was visualized and photographed with illumination under UV light using a photodocumentation hood (Fisher Scientific, Pittsburgh, PA, USA) equipped with a Polaroid 667 film with an orange filter (Kodak, Rochester, NY, USA).

Statistical analysis

The results were expressed as mean ± SD of different groups. The differences between the mean values were evaluated by ANOVA followed by Student's "t" test using Minitab 12 computer program (Minitab Inc., State Collage, P.A).

3. Results

Histological observations

Figure (1) showed the histological structure of the liver of control rat. Liver of animals administered with O. *basilicum* appeared with normal structure. On the other hand, Liver of rats treated with CCl_4 for two weeks showed impairment of the normal structural organization of the hepatic lobules and sinusoidal spaces were enlarged. Intrahepatic veins, central and portal were dilated and congested (Fig. 2). Leucocytic infiltrations were observed (Fig.3). Liver sections prepared from rats 4 weeks post-treatment with CCl_4 revealed that a considerable number of hepatic cells were damaged and lost their characteristic appearance while others showed marked cytoplasmic vacuolization which was so extensive in some cells to the extent that only slight remnants of the cytoplasmic mass cells - frequently forming a narrow peripheral rim was left (Fig.4). The nuclei of these cells were pyknotic. In addition, congestion of the intrahepatic blood vessels and inflammatory leucocytic infiltrations were observed. The histopathological changes of the liver were more increased after 6 weeks and the liver cells were degenerated and suffered from micro and macrovesicular steatosis (Fig.5). Examination of liver sections obtained from rats treated with both CCl_4 and O. *basilicum* for 2 and 4 weeks revealed gradual restoration of the normal structure of liver tissues. A rare leucocytic infiltration was observed, but the central as well as the portal veins were congested (Fig.6). A large number of binucleated hepatocytes were observed. After 6 weeks, the liver tissue appeared normal and fatty infiltrations was absent in the examined specimens (Fig.7).

Biochemical results

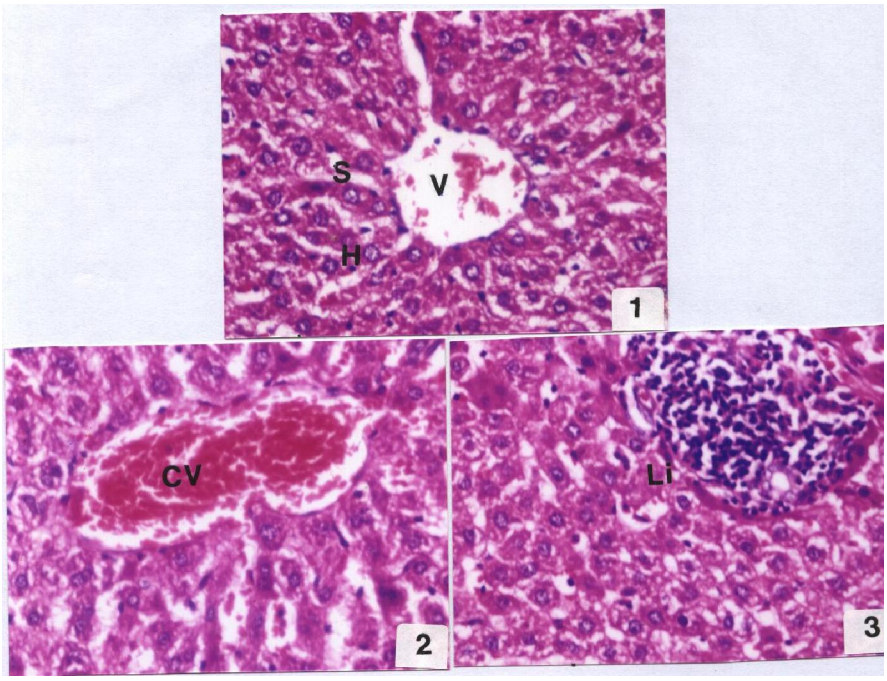
Treatment with CCl_4 for 6 weeks caused a highly significant elevation ($P<0.05$) in the activity of ALT, AST and ALP as compared to those of the control animals. All these parameters were restored to near normal values in rats treated CCl_4 and *O. basilicum* (Figs.8-10).Both control and animals given *O. basilicum* showed no significant differences in serum activity of ALT, AST and ALP. Administration of CCl_4 to rats caused significant increase in cholesterol and triglycerides compared with animals of control groups. Animals treated with both CCl_4 and *O. basilicum* extract showed reduction in their sera level of cholesterol and triglycerides in comparison of those given CCl_4 (Figs.11&12). Figures 13 and 14 showed that treating animals with CCl_4 induced significant increase in serum HDL and LDL concentrations after 4 and 6 weeks post - treatment compared with control group. On the other hand, animals treated with CCl_4 and *O. basilicum* extract had a noticeable increase in the concentration of these parameters compared with animals received CCl_4 alone.

Biochemical features of apoptosis

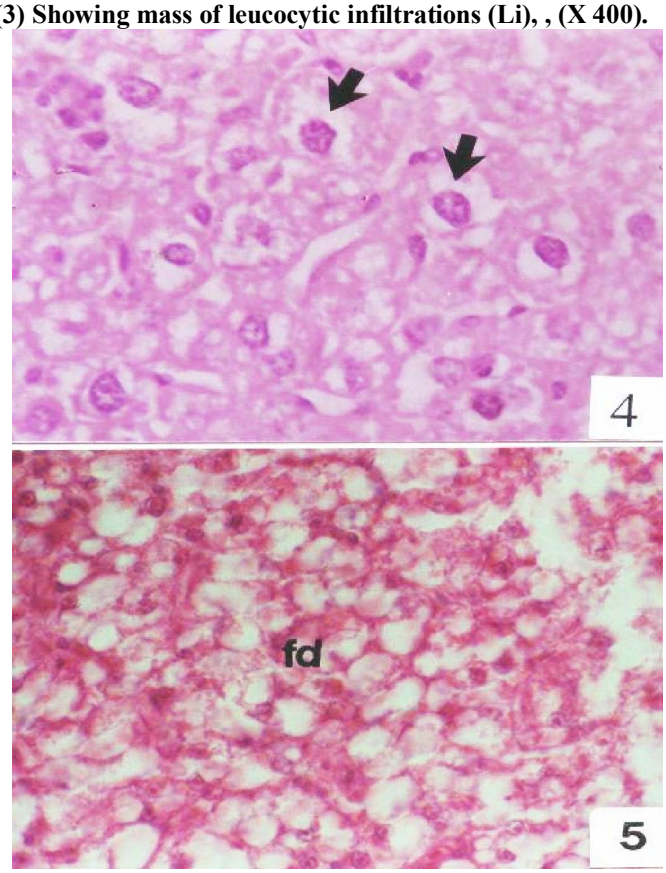
Administration of carbon tetrachloride for 6 weeks induced fragmentation of DNA in rat livers (Fig.15). The total optical density of released DNA was 128 when compared with controls (table 1). Animals treated with *O. basilicum* were not display any increased in fragmented DNA and the total optical density was in normal range.Fragmentation of DNA was repaired by shared administration with both *O. basilicum* and carbon tetrachloride for 6 weeks as rosemary significantly decreased the total optical density of released DNA with value of 25 when compared with CCl_4 treated group.

4. Discussion

Results obtained in the present work indicated that CCl_4 induced histological and biochemical alterations in liver of albino rats. Concerning the histological effects, liver of CCl_4-treated animals showed many degenerative changes including cytoplasmic vacuolization of the hepatocytes, fatty infiltrations, leucocytic infiltrations, congestion of blood vessels, and fibrosis. Similar results were obtained by **Sakr *et al.* *(2010)*** in albino rats intoxicated with CCl_4. Moreover, the current results are in accordance with those of **Sreelatha *et al.*(2009)** and **Lodhi *et al.*(2009)** who reported that liver injury including marked alteration of the entire liver structures with degenerative changes were observed after CCl_4 administration. Fatty infiltrations were observed in liver of CCl_4 treated rats. In agreement with this result **Qiu *et al.* (2005)** and **Panovska *et al.* (2008)** reported that CCl_4 caused extensive liver necrosis and fatty changes .**Brody *et al* .(1961)** attributed the fatty changes in the liver to excessive mobilization of free fatty acids from the fat depots induced by the lipolytic effects of the increased circulating catecholamines and the centrilobular necrosis to the catecholamines-induced decrease in hepatic flow. Liver fibrosis was observed after 6 weeks of treating rats with CCl_4. **Qiu *et al.* (2005)** reported that CCl_4 caused centrilobular necrosis followed by fibrosis.**Nan *et al.*(2002)** mentioned that CCl_4 is the most widely used chemical for inducing liver fibrosis.



Figs. 1-3: (1) Section of liver of a control rat showing hepatocytes (H), central vein (V), sinusoids (S) and Kupffer cells (K), (2) section of liver of ccl_4-treated rat after two weeks showing congested and enlarged central vein (CV), (3) Showing mass of leucocytic infiltrations (Li), , (X 400).



Figs 4-5: (4).Liver section of ccl_4-treated rat for 4 weeks showing cytoplasmic vacuolization of the hepatocytes (arrows).(5), Specimen obtained from a rat treated with ccl_4 for 6 weeks showing fat droplets (fd) of different sizes,(X 400).

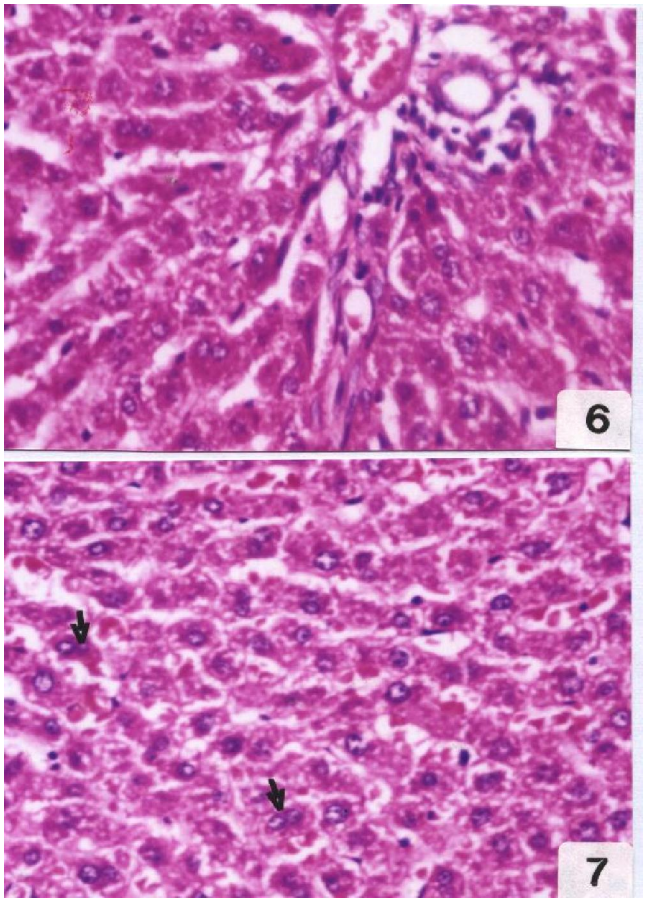


Fig 6-7: (6). Liver section of a rat treated with ccl_4 and ocemium for 4 weeks showing congested portal vein (P), (7) Specimen obtained from a rat treated with ccl_4 and ocemium showing an obvious degree of improvement with large number of binucleated cells (arrows), (X 400).

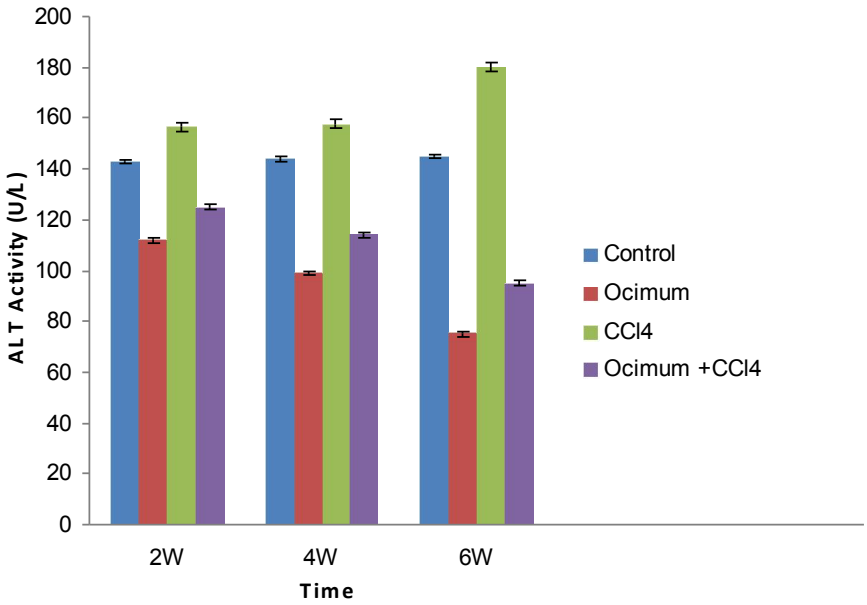


Fig.8. Change in ALT activity in different experimental groups

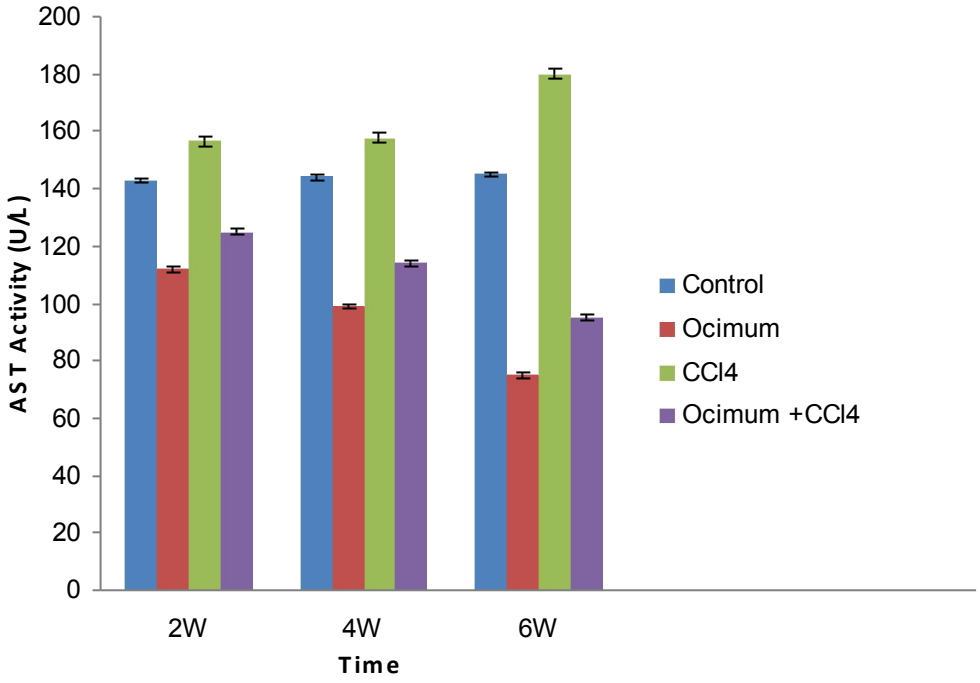


Fig.9. Change in AST activity in different experimental groups

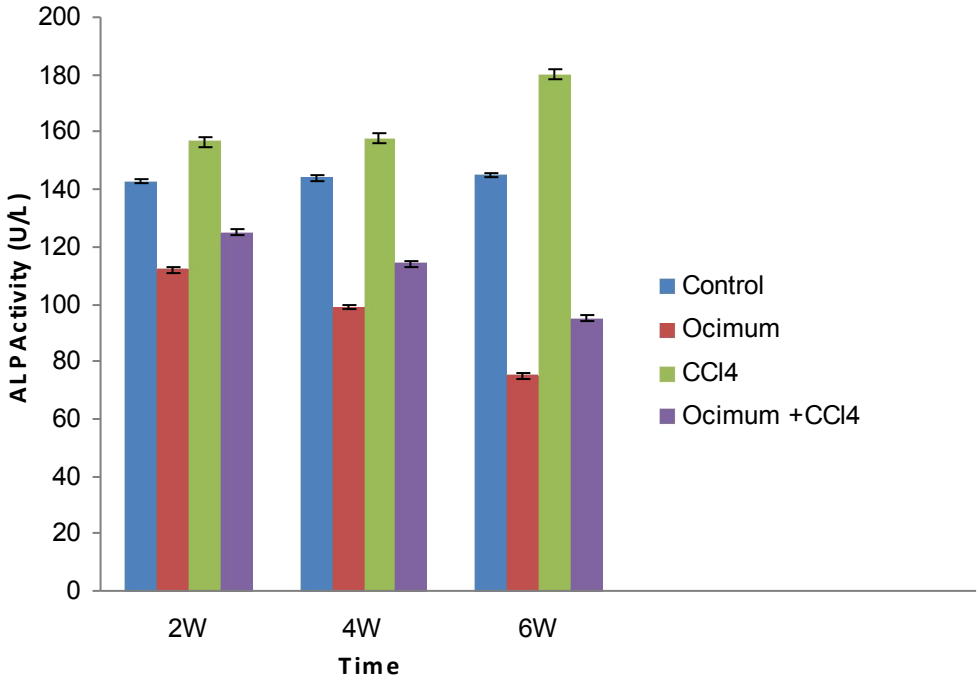


Fig.10. Change in ALP activity in different experimental groups

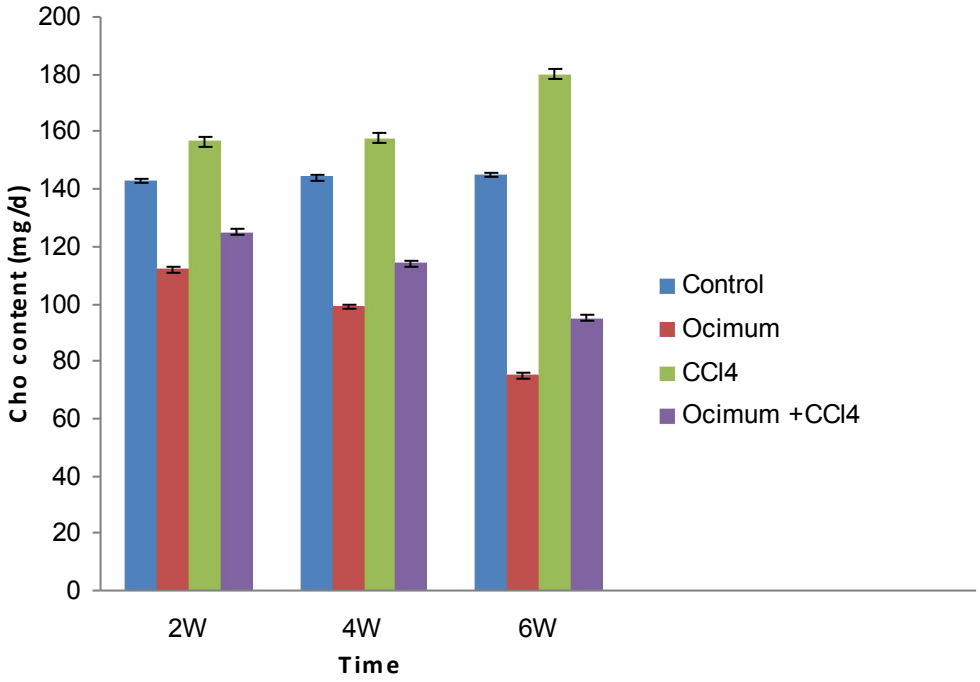


Fig.11. Change in serum cholesterol in different experimental groups

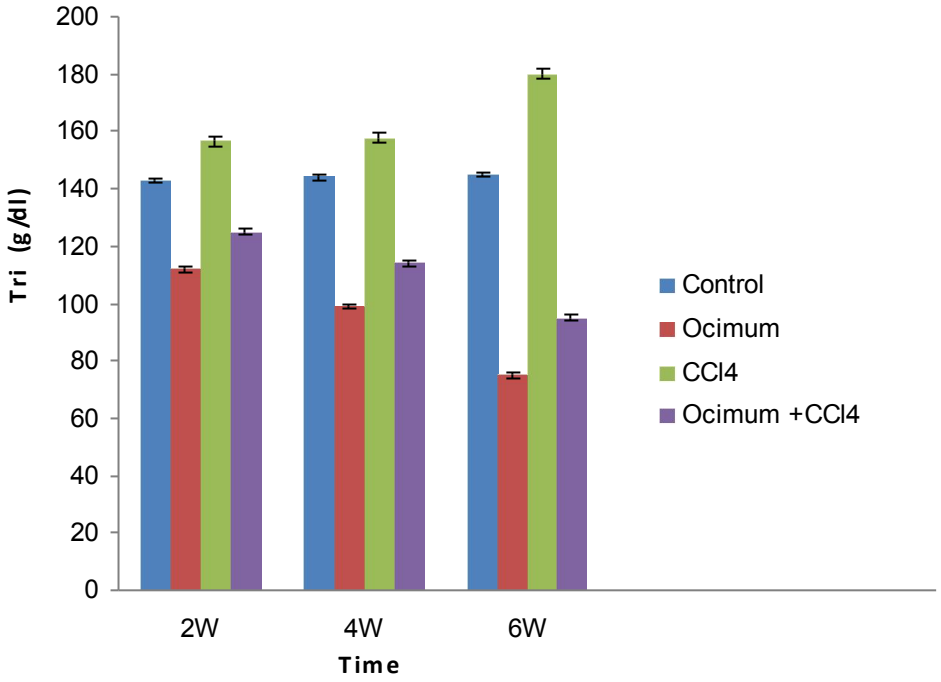


Fig.12. Change in serum triglycerides in different experimental groups

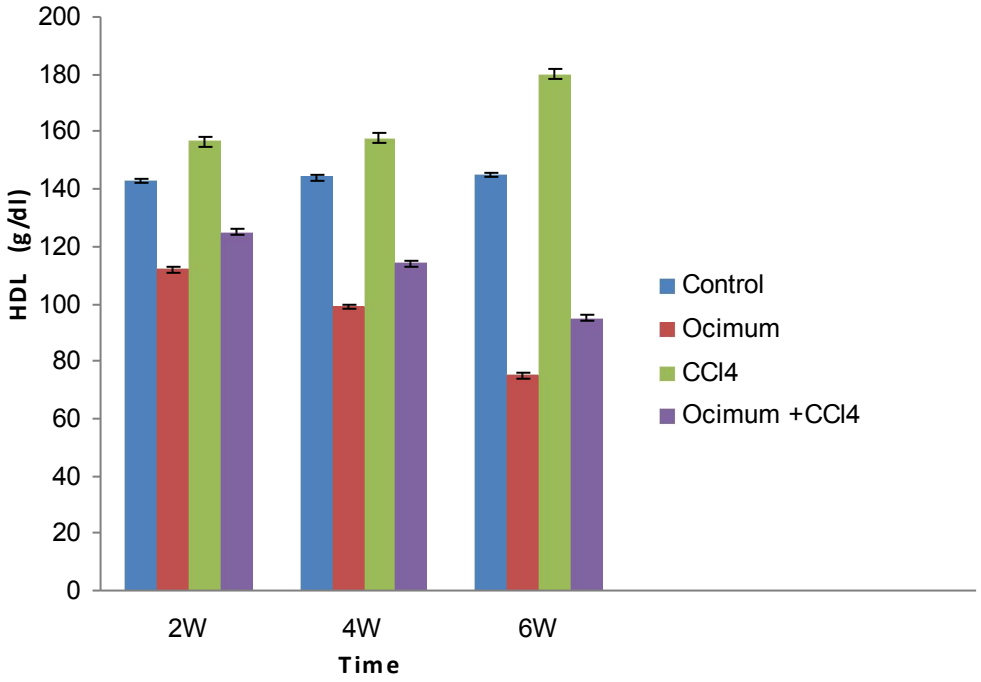


Fig.13. Change in HDL in different experimental groups

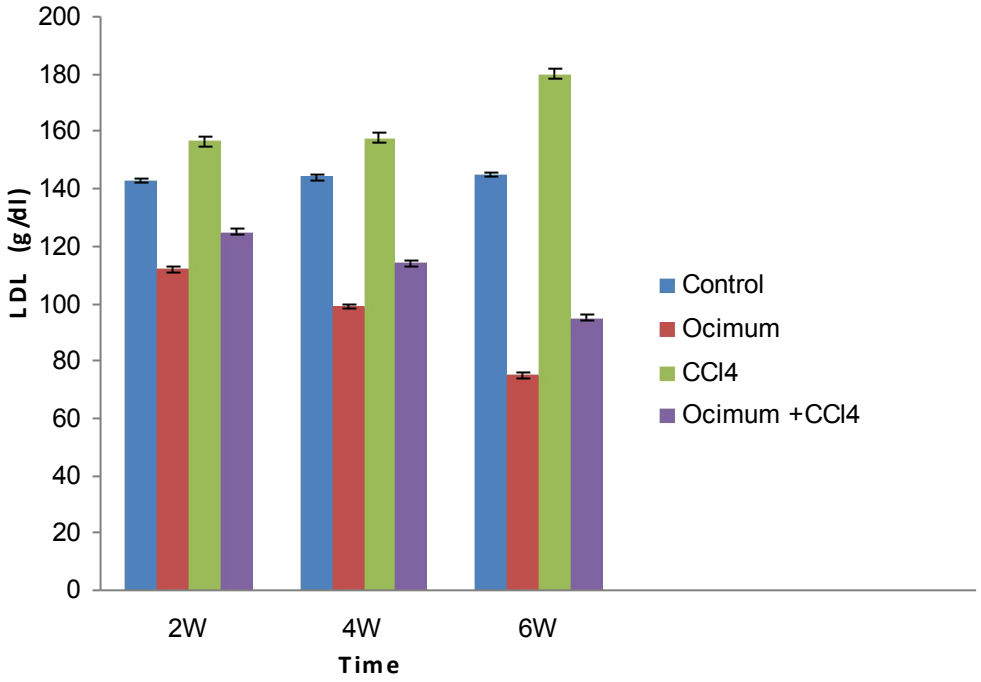


Fig.14. Change in LDL in different experimental groups

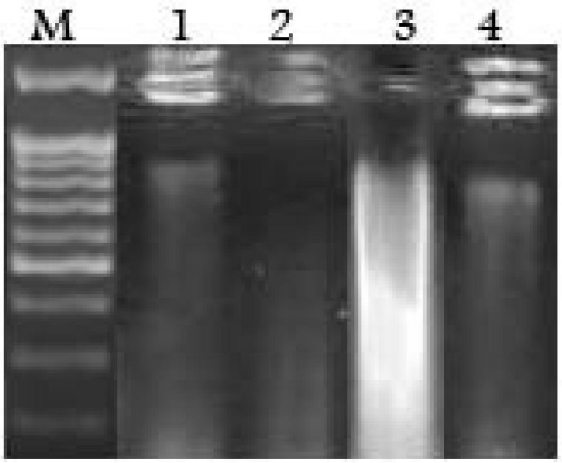


Fig.15. Gel electrophoresis of hepatic DNA M: standard lane, Lane 1: control, lane 2 ocemium, Lane 3: ccl_4 group,, lane 4: ccl_4 and ocemium group.

Table (1): Changes in values of the total optical density of both intact and fragmented DNA induced in liver of rat.

Total optical density	Control group.	Ocimum group	CCL4 group	CCL4+ Ocimum group
Intact DNA	61	58	32	53
Fragmented DNA	**5**	**7**	128	25

CCl_4 was found to induce apoptosis as represented by DNA fragmentation. This result came in agreement with **Castro *et al.* (1993)** who reported that CCl_4 induced necrosis and DNA fragmentation in Sprague-Dawley male. Rats. **Shi *et al.*(1998)** proved that carbon tetrachloride poisoning induced DNA fragmentation, apoptosis and necrosis in rat liver by immunohistochemical labeling of nuclear DNA fragmentation, flow cytometry and gel electrophoresis .

Significant increase in ALT, AST and ALP levels of sera of CCl_4 treated rats was recorded in the present study. In agreement of this result, **Wang *et al.* (1996)** observed that a single i.p. injection of CCl_4 caused an increase in ALT and AST. Pablo and **Yesenia (2003)** found that liver injury induced by CCl_4 in Wistar rats was accompanied by elevation in serum level of ALT,AST and ALP. Increase in triglycerides and cholesterol were recorded in sera after exposure to CCl_4. Similarly, **Torres-Duran *et al.* (1999)** reported that CCl_4 caused elevation in LDL, HDL, triglycerides and cholesterol.

Oxidative stress is a state of redox imbalance caused by increased reactive oxygen species (ROS) generation and decreased antioxidant capacity. Administration of CCl_4 is an established experimental model of severe toxic liver injury involving generation of oxidative stress. It has been reported that exposure to CCl_4 induces oxidative stress in rats (**Sharma *et al.*,1994**). Oxidative damage primarily occurs through production of reactive oxygen species, including CCl_3 and CCl_3O_2 radicals that subsequently react with biological molecules as well as causing damage to membranes (**Singh *et al.*,1998).** A decrease in the level of antioxidant enzymes and an increase in lipid peroxidation level were recorded after CCl_4 administration (**Campo *et al.*, 2004**).The increase in lipid peroxidation in the liver following exposure to CCl_4 may lead to membrane damage resulting in damage of liver cells. The increase in ALT, AST and ALP is the end results of this phenomenon.

The present findings demonstrated that *O.basilicum* improve the histological changes and increased liver function enzyme activity induced by CCl_4. This indicated the effectiveness of *O.basilicum* in prevention of CCl_4 hepatotoxicity. The hepatoprotective effects of *O.basilicum* have been

shown in studies on experimental liver damage. **Yamamoto *et al.*, (2005)** proved that ocimum suppressed hepatic fibrosis and protected liver against parenchymal damage induced by CCL_4.Significant hepatoprotective effects were obtained by ethanolic extract of leaves of *O. basilicum* against liver damage induced by H_2O_2 and CCl_4 in goat as evidenced by decreased levels of antioxidant enzymes. The extract also showed significant anti lipid peroxidation effects in vitro, besides exhibiting significant activity in superoxide radical and nitric oxide radical scavenging, indicating their potent antioxidant effects (**Meera *et al.*, 2009**). **Adhvaryu *et al.*, (2007)** reported that *O. sanctum* have hepatoprotective and immunomodulatory effects on liver injury and immunosuppression induced by Isoniazid , Rifampicin and Pyrazinamide in guinea pig. It has been shown that 2% of dried *O. sanctum* leaf powder supplemented in the diet can lower serum lipid profile and partially protect the liver in diabetic rats (**Suanarunsawat and Songsak, 2005**). It has also been shown that *O. sanctum* leaf extracts can protect the liver from heavy metals (**Sharma *et al.*, 2002**) and prevent isoproterenal induced myocardial necrosis in rats (**Sood *et al.*,2005**). O.basilicum treatment attenuated serum lipid profile. This may be due to the anti-hyperlipidemic action of components of *O.basilicum* leaves. **Suanarunsawat *et al.* (2009)** mentioned that the anti-hyperlipidemic activity of *O.basilicum* may be due to the suppression of liver lipid synthesis.

Zhang *et al.*(2009) reported that the main components of *O. basilicum* are : linalool (29.68%) , (Z)-cinnamic acid methyl ester (21.49%) , cyclohexene (4.41%) , alpha- cadinol (3.99%) , 2,4-diisopropenyl-1-methyl-1-vinylcyclohexane (2.27%), 3,5-pyridine-dicarboxylic acid, 2,6-dimethyl-diethyl ester (2.01%), beta-cubebene (1.97%), guaia-1(10),11-diene (1.58%), cadinene (1.41%) (E)-cinnamic acid methyl ester (1.36%) and beta-guaiene (1.30%). **Lee and Scagel (2009)** reported that the presence of chicoric acid (dicaffeoyltartaric acid) was the major phenolic compound, in basil leaves. *O.basilicum* is rich source of flavonoids which have been shown to possess various biological properties related to antioxidant mechanisms. **Dasgupta *et al.*, (2007)** reported that *O.basilicum* increased the activity of xenobiotic metabolizing phase 1 and phase 11 enzymes, elevating antioxidant-enzyme response by increasing significantly the hepatic glutathione reductase, superoxide dismutase, and catalase activities, increasing glutathione content and decreasing lipid peroxidation and lactate dehydrogenase activity in the liver of mice. **Chinnasamy *et al.*, (2007)** oreported that the protective action of ocimum was attributed to its antioxidant action. They added that this protection may be also due to anti-inflammatory property of ocimum which reduces formation, release, and activity of inflammatory mediators such as cytokines, histamine, prostaglandins, and leukotrienes. **Suanarunsawat *et al.* (2009)** reported that *O. sanctum* leaf have lipidlilowering effect and antioxidant activity in rats fed with a high cholesterol diet. It is concluded from the present work that the hepatoprotective of O. *basilicum* may be attributed to the antioxidant activity of its flavonoids.

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The enhancement of the anti inflammatory effect of Caffeine on Green tea extract and EGCG on obese rats

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Abstract: Obesity is linked directly with the increased acute phase protein like c-reactive protein (CRP)and increased production of some cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) as a result of an inflammatory signaling pathway. The health benefits of green tea are related to its catechins particularly epigallocatechin-3-gallatel (EGCG) .Green tea contains caffeine which stimulate thermogenesis and fat oxidation. The aim of this study was to evaluate the effect of caffeine on EGCG –and green tea extract–administered obese rats induced by high-fat diet. The animals were divided into two groups. The first group served as healthy control group. The second group was fed high fat –diet for 8 week and was divided into 6 subgroups. IL-6, CRP and TNF-α showed a high significant increase ($p<0.01$) at zero level and after 5 and 9 weeks of obesity induction as compared to control group. The results showed a decrease in proinflammatory cytokines effect of EGCG, green tea extract and caffeine on TNF-α after 5 weeks ($P<0.051$,$P<0.039$,$P<0.002$), for IL6were($P<0.001$,$P<0.001$,$P<0.000$) , and for CRP($P<0.066$,$P<0.007$, $P<0.002$) respectively The 9 weeks treatment by EGCG, Green tea extract and caffeine on TNF-α ($P<0.312$, $P<0122$, $P<0.057$) ,on IL-6 ($P<0.155$,$P<0.029$,$P<0.033$), on CRP ($P<0.387$ $P<0.168$, $P<0.088$). The addition of caffeine to green tea showed enhancement of the effect with decrease of TNF-α , IL-6 and CRP after 5 weeks ($P<0.03$, $P<0.001$ $P<0.034$) respectively where after the 9 weeks all the parameters reversed back to normal .The addition of caffeine to EGCG after 5 weeks showed enhancement of the effect on TNF-α, IL-6 and CRP |($P<0.04$, $P<0.011$, $P<0.066$) respectively, at the end of the 9 weeks all the inflammatory parameters return to normal levels. As conclusion, the addition of caffeine to EGCG or green tea extract enhance their anti-inflammatory effects on the low grade inflammation state a companied with obesity by decreasing the levels of TNF-α,IL-6 and CRP.

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1. Introduction

Obesity is the direct result of the imbalance between the energy intake and energy expenditure (Ulijaszek 2008). Over the past several decades , there has been an alarming increase in the prevalence of obesity both in high-income as well as middle-and low-income countries (Lobstein et al. 2004) Several studies have shown that experimental animal and human obesity associated with the development atherosclerosis and increased prevalence of inflammatory diseases (Silver et al. 2007) The link between the obesity and the inflammation has been reported by the increase in plasma levels of several pro-inflammatory molecules including cytokines such as tumer necrosis factor-alpha (TNFα) and interleukin-6(IL-6) which may have local effects on adipose tissue physiology and also systemic effects in other tissues (Bastard etal.2006). Obesity is also closely associated with chronic inflammatory state resulted from increased acute phase proteins and other mediators and activation of a network of inflammatory signalling pathway. C-reactive protien (CRP) is a major human acute phase protein largely synthesized in hepatocytes following inflammatory stimuli (Black et al. 2004). Cytokines particularly IL-6 has been found to induce hepatic CRP synthesis in primary hepatocytes and hepatoma cells at the transcriptional level (Reza et al.2009) .TNF-α is a multifunctional circulating proinflammatory cytokine found to play a major role in the cytokine cascade as it stimulate the synthesis of other cytokines like IL-6 which is a central mediator of the acute phase protein response and the primary determinant of CRP production there by it contribute to the maintenance of chronic low grade-inflammation involved in the progression of obesity and its associated of co morbidities(Harris et al.2008). it has been reported that TNFα was overexpressed in adipose tissue of obese animal and human and its concentration is reduced after weight loss (Ziccardi et al.2002)

The health benefits of green tea were reported many of these benefits of green tea are related to its catechins particularly epigallocatechin-3-gallatel(EGCG) . There evidences from in vitro and animals studies on the underlying mechanisms of green tea catechins and their antiobesity and ant-

inflammatory effects (Sabu et al.2010).These mechanisms may be related to certain pathways such as through the modulation of energy balance ,endocrine system, food intake, lipid and carbohydrate metabolism and redox status (Yang et al.2001).Oral administration of EGCG in aged rats fed a high –fat diet was also shown to significantly decrease CRP and TNF-α versus the unsupplemented group (Senthl et al.2009). Green tea contains caffeine which is a member of methylxanthine family of drugs that may stimulate thermogenesis and fat oxidation through inhibition of phosphodiesterase ,(Belza et al.2007).Caffeine has been previously utilized in therapies for weight loss,due to claimed action of amplifying the lipolytic effect of ephedrine(Diepvens and Westertrep 2007).

The increased and prolonged sympathetic stimulation of thermogenesis by the interaction between EGCG and caffeine could be of value in assisting the management of obesity by suppressing the production of proinflammatory cytokine TNF-α and IL-6 (Dulloo et al.2006).
It was appeared that, Nature is, in fact ,the richest botanic pharmacy created for living beings and the scientific efforts must certainly be more oriented towards the discovery of nature in future. Thus, the present study aimed to investigate the effect of caffeine on EGCG –and green tea extract –administered in obese rats tacking some pro inflammatory cytokines in account.

2. Materials and Methods

Materials:

1. Green tea extract was obtained from the leaves of the Chinese green tea obtained from a local market.
2. Caffeine was obtained from Sigma chemical company U.S.A.
3. E.G.C.G was obtained from Sigma chemical company U.S.A.
4. Green tea extract, was obtained from boiling the chine's green tea leaves.
5. The Stock solution of Caffeine and EGCG were prepared by dissolving in sterile double distilled H2Oat a final concentration of 100Mm. Stock solutions were aliquots and kept at -20C for long term storage.

Animals and drug administration:

From four to six weeks old male albino rats (Sprague Dawely strains), about 150 Dokki, Giza, Egypt. The environmental conditions were properly standardized with a 12- hours light cycle and a constant temperature of 20ºC and humidity of 48%.

Animals were fed on a standard laboratory pellets and tap water ad libitum. All experimental procedures conformed to the National Health and Medical Research Council guidelines and were approved by an institutional animal ethics committee.
The animals were divided into two groups. The first group served as healthy control group and composed of 20 rats.

Obesity was induced in the second group by feeding high fat –diet for 8 weeks. The second group (obese rats) was divided into 6 subgroups each composed of20 rats2-1: the first subgroup served as obese positive control group continue to feed high fat-diet and supplied distilled water through stomach lavage.
2-2: the second group was supplied with green tea extract (4.86mg/200gm.body weight) through stomach lavage.
2-3: the third group were supplied with epigallocatchin-3-gallate (EGCG)(1.44mg/200gm. Body weight) through stomach lavage.
2-4: the fourth group was supplied with caffeine (2.7 mg/200gm body weight) through stomach lavage.
2-5: the fifth group were supplied with a mixture of caffeine (2.7mg/200gm body weight)and green tea extract (4.86mg/200gm body weight)through stomach lavage.
2-6: the six group were supplied with a mixture of caffeine (2.7mg/200gm body weight) and EGCG (1.4mg/200gm body weight) through stomach lavage.
The supplement dose of the treatment was given according to Paget and Barnas (1964), for rats 200gm = human dose *0.018 by intragastric lavage using stomach tube.

Sample collections:

The fasting blood samples were collected from the retro orbital plexus of rats (Schemer, 1967), under diethyl anesthesia by clean heparinized capillary tubes and were left to clots, then centrifuged at 5000 rpm for 10 minutes to separate sera which in turn were used for the determination of different biochemical parameter. Blood samples were collected after induction of obesity (zero level), 5weeks after prevalence of obesity and after 9 weeks of prevalence of obesity.

Biochemical analysis:

Serum TNF-α was determined by using high sensitivity ELISA, enzyme –linked immunosorbent assay for quantitative detection of rat tumor necrosis factor -α from Immunotech A Beckman culture company. Sensitivity 5pg/ml with intra-assay coefficient of variation ranged between 1.6 and 10 % and inter-assay variation coefficient ranged between 5.4 and 12.8%

Rat IL-6 was determined by using Platinum ELISA, enzyme –linked immunosorbent assay for quantitative detection of rat IL-6 from Bioscience Bender Medsystem , Vienna. Sensitivity 12pg/ml

with intra-assay coefficient of variation was<5% and inter-assay coefficient of variation <10%.

Rat CRP was determined by using a solid phase capture sandwich enzyme-linked immunosorbent assay ELISA kit, from Assay Pro Company .sensitivity less than 70ng /ml with intra-assay coefficient of variation 5.5% and inter-assay coefficient of variation 7.6%

Statistical analysis;

The results were expressed as mean ± standard deviation .statistical analysis of differences between means were performed using student "t" test (Sendecor and Cochran, 1967)

3. Results

TNF-α at zero level (obese rats) showed a high significant increase *(p<0.001)* and highly significant increase *(p<0.000)* after 5 weeks and after 9 weeks of induction of obesity as compared to control group (Table1).

IL-6 at zero level (obese rats)showed a high significant increase *(p<0.001)* and high significant increase *(p<0.002)* after 5 weeks and after 9 weeks of induction of obesity as compared to control group (Table1).

CRP levels showed high significant increase at zero level (obese rats) *(p<0.014)* and after 5 weeks and *after* 9 weeks of induction of obesity *(p<0.002(p<0.001) respectively as* compared to control group (Table1).

After 5 weeks treatment with EGCG, TNF-α showed high a significant decrease *(p<0.05)* as compared to normal control .There is no statistical difference between the 9 weeks result and normal control (*p<0.312) (*Table2).

IL-6 showed a high significant decrease *(p<0.001)* after 5 weeks of treatment with EGCG and with no significant difference (*p<0.155)* after 9 weeks of treatment as compared to control group (Table2). CRP levels showed no significant difference from control after 5 weeks of treatment *(p<0.066)* and showed no significant difference after 9 weeks of treatment with EGCG *(p<0.387)* as compared to control group (Table 2).

TNF-α showed high significant decrease after 5 weeks *(p<0.020) of caffeine treatment* and showed no statistically significant difference *(p<0.06)* after 9 weeks of treatment as compared to control group (Table3).IL-6 showed highly significant decrease after 5 weeks of treatment (p<0.000) with caffeine and high significant decrease (p<0.033) after 9 weeks of treatment as compared to control group Table (3). Treatment with caffeine showed high significant decrease of CRP after 5 weeks of treatment (p<0.002) in comparison to control. It decreased after 9 weeks of treatment (p<0.088) put showed no significant difference when compared to control group (Table 3).

Treatment with green tea extract showed high significant decrease of TNF-α after 5 weeks (p<0.039) when compared to control and showed no statistical significant difference (p<0.122) after 9 weeks of treatment as compared to control group (Table4).

IL-6 showed high significant decrease after 5 weeks of treatment (p<0.001) and high significant decrease (p<0.03) after 9 weeks of treatment as compared to control group (Table4). CRP level showed high significant decrease after 5 weeks of treatment with green tea extract *(p<0.007)* and showed no statistical significant difference after 9 weeks of treatment *(p<0.168)* as compared to control group (Table 4).

The addition of Caffeine to green tea extract showed enhancement of the effect with a significant decrease of TNF-α after 5 weeks of treatment (P<0.03),with no significance difference from control after9 weeks of treatment(P<0.06) (Table5).

Where IL-6 showed a high decrease after 5weeks of treatment with the caffeine green tea mixture (P<0.001)and no statistical difference from normal control after 9 weeks of treatment(P<0.11) (Table5).CRP showed a significant decrease after treatment with mixture of caffeine and green tea extract (P<0.034) with no statistical difference than the normal control (P<0.150) (Table 5).

The administration of a mixture of EGCG and caffeine cause decrease in TNF-α after 5 weeks of treatment (P<0.04) where after 9 weeks of treatment TNF-α showed no significance difference from normal control (P<0.606)(Table (6).The level of IL-6 after 5 weeks of administration of the mixture showed significant decrease (P<0.011) with no significant difference from normal control after 9 weeks of treatment (Table6).The administration of the EGCG and caffeine mixture cause a high significant decrease of CRP (P<0.066) where after 9 weeks of administration the level of CRP was with no statistical difference compare to control (P<0.497) (Table 6).

The comparison of the effect of the obese, EGCG,, Caffeine, Green tea extract ,Caffeine plus Green tea extract and caffeine plus EGCG on TNF-α after 5 weeks of treatment showed an enhancement effect of caffeine on green tea and EGCG ,(121mg/ml), (66mg/ml), (80mg/ml), (98mg/ml), (75mg/ml)(63mg/ml) respectively (Fig.1)

The comparison of the effect on TNF-α after9 weeks of treatment showed enhancement of caffeine on green tea and EGCG obese(138mg/ml), EGCG(37mg/ml),green tea extract(51mg/ml),

caffeine (43mg/ml),caffeine plus green tea extract (38mg/ml) and caffeine plus EGCG (33mg/ml) (Fig2).

The administration of caffeine in mixture with green tea or EGCG cause enhancement ot there effect onIL-6 in comparison to other groups after 5 and 9 weeks (Fig3 and fig 4) respectively.

The effect of administration of obesity, EGCG, green tea extract, caffeine in comparison to administration of mixture of caffeine plus green tea extract or caffeine mixed with EGCG on CRP after 5 weeks (4.8mg/ml,2.8mg/ml ,3.4mg/ml, 3.06mg/ml, 2.85mg/ml and 2.7mg/ml) respectively and after9 weeks (5.1mg /ml,2.36mg /ml,2.74mg /ml,2,59mg /ml,2.47 mg /ml ,2.59mg /ml and 2.30mg /ml) respectively were represented in (fig 5 ,and fig 6).

Table (1) Comparison of pro-inflammatory cytokine between obese groups without treatment compared to control group.

	control	Obese at zero level	Obese after 5 weeks	Obese after 9 weeks	Obese at zero level VS control	Obese after 5 weeksVS control	Obese after 9 weeks VS control
TNF-α mg\ ml	32.5 ±2.04	105.63 ±12.48	120.88 ±11.61	137.75 ±3.4	P < 0.001	P < 0.000	P < 0.000
IL-6 mg\ ml	197.5 ±28.75	407.5 ±17.07	432.75 ±29.5	486.75 ±26.17	P < 0.001	P < 0.002	P < 0.000
C R P mg\ ml	2,037 ±0.39	4.3 ±0.49	4.8 ±0.21	5.1 ±0.23	P < 0.014	P < 0.002	P < 0.001

- Data are expressed as mean ± standard deviation.
P< 0.05 Significant P< 0.001 High significant P< 0.000 highly significant - .

Table (2): The effect of EGCG alone on the pro-inflammatory cytokine at zero level and after 5 and 9 weeks of treatment in obese rats compared to the control group.

	control	EGCG at zero level	EGCG after 5 weeks	EGCG after 9 weeks	EGCG at zero level VS control	EGCG after 5 weeksVS control	EGCG after 9 weeks VS control
TNF-α mg\ ml	32.5 ±2.04	105.63 ±12.48	65.50 ±20.74	36.75 ±5.62	P < 0.001	P < 0.051	P < 0.312
IL-6 mg\ ml	197.5 ±28.75	407.5 ±17.07	346.5 ±18.5	220 .00 ±6.9	P < 0.001	P < 0.001	P < 0.155
C R P mg\ ml	2,037 ±0.39	4.30 ±0.49	2.80 ±0.58	2.36 ±0.36	P < 0.014	P < 0.066	P < 0.387

- Data are expressed as mean ± standard deviation.
- P< 0.05 Significant P< 0.001 High significant P< 0.000 Highly significant-

Table (3): The effect of caffeine alone on the pro-inflammatory cytokine levels after 5 and 9 weeks of treatment in obese rats compared to the control group.

	Control	caffeine at zero level	caffeine after 5 weeks	caffeine after 9 weeks	caffeine at zero levelVS control	caffeine after 5 weeksVS control	caffeine after 9 weeks VS control
TNF-α mg\ ml	32.5 ±2.02	105.63 ±12.48	80.125 ±20.56	42.88 ±7.78	P < 0.001	P < 0.020	P < 0.057
IL-6 mg\ ml	197.5 ±28.75	407.5 ±17.07	374.5 ±28.8	260.05 ±24.71	P < 0.001	P < 0.000	P < 0.033
C R P mg\ ml	2,04 ±0.39	4.3 ±..48	3.06 ±0.27	2.47 ±0.23	P < 0.014	P < 0.002	P < 0.088

- Data are expressed as mean ± standard deviation.
P< 0.05 Significant P< 0.001 High significant P< 0.000 Highly significant.

Table (4): The effect of the extract of green tea alone on the pro-inflammatory cytokine levels after 5 and 9 weeks of treatment in obese rats compared to the control group.

	control	Extract at zero level	Extract after 5 weeks	Extract after 9 weeks	Extract at zero level VS control	Extract after 5 weeksVS control	Extract after 9 weeks VS control
TNF-α mg\ ml	32.5 ±2.04	105.63 ±12.48	97.63 ±35.3	50.63 ±15.35	P < 0.001	P < 0.039	P < 0.122
IL-6 mg\ ml	197.5 ±28.75	407.5 ±17.07	390.5 ±19.25	263.5 ±13.9	P < 0.001	P < 0.001	P < 0.029
C R P mg\ ml	2,037 ±0.39	4.30 ±0.49	3.41 ±0.79	2.74 ±0.39	P < 0.014	P < 0.007	P < 0.168

- Data are expressed as mean ± standard deviation.
- P< 0.05 Significant P< 0.001 High significant P< 0.000 Highly significant

Table (5): The effect of the extract of green tea with caffeine in a combination on the pro-inflammatory cytokine levels after 5 and 9 weeks of treatment in obese rats compared to the control group.

	control	(extract-caffeine) at zero level	(extract-caffeine) after 5 weeks	(extract-caffeine) after 9 weeks	(extract-caffeine) at zero level VS control	(extract-caffeine) after 5 weeksVS control	(extract-caffeine) after 9 weeks VS control
TNF-α mg\ ml	32.5 ±2.04	105.63 ±12.48	74.63 ±21.61	37.5 ±2.88	P < 0.001	P < 0.031	P < 0.066
IL-6 mg\ ml	197.5 ±28.75	407.5 ±17.07	371.25 ±15.01	229.00 ±16.85	P < 0.001	P < 0.001	P < 0.110
C R P mg\ ml	2,037 ±0.39	4.30 ±0.49	2.85 ±0.42	2.95 ±0.40	P < 0.014	P < 0.034	P < 0.150

- Data are expressed as mean ± standard deviation.
- P< 0.05 Significant P< 0.001 High significant P< 0.000 Highly significant

Table (6): The effect of EGCG with caffeine in a combination on the pro-inflammatory cytokine levels after 5 and 9 weeks of treatment in obese rats compared to the control group.

	control	(EGCG - caffeine) at zero level	(EGCG - caffeine) after 5 weeks	(EGCG - caffeine) after 9 weeks	(EGCG - caffeine) at zero level VS control	(EGCG - caffeine) after 5 weeksVS control	(EGCG - caffeine) after 9 weeks VS control
TNF-α mg\ ml	32.5 ±2.04	105.63 ±12.48	62.63 ±16.6	33.13 ±0.75	P < 0.001	P < 0.041	P < 0.606
IL-6 mg\ ml	197.5 ±28.75	407.5 ±17.07	334.5 ±25.52	172.5 ±9.7	P < 0.001	P < 0.011	P < 0.248
C R P mg\ ml	2,037 ±0.39	4.30 ±0.49	2.70 ±0.19	2.3 ±0.43	P < 0.014	P < 0.066	P < 0.497

- Data are expressed as mean ± standard deviation.
- P< 0.05 Significant P< 0.001 High significant P< 0.000 Highly significant

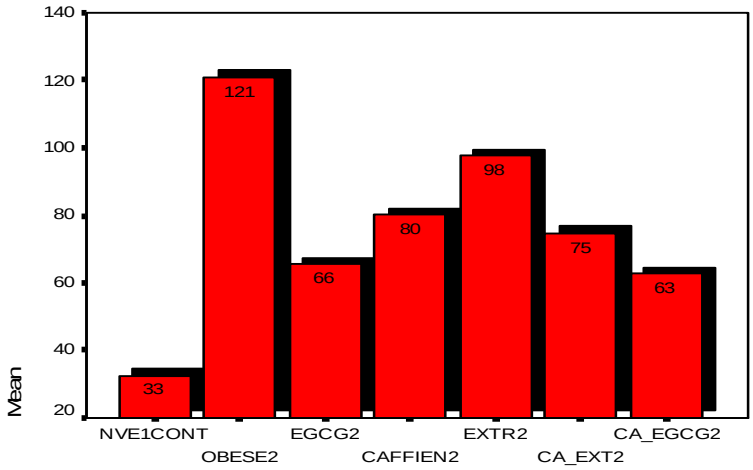

Fig (1): comparison of TNF-α between different groups and normal group 5weeks of treatment

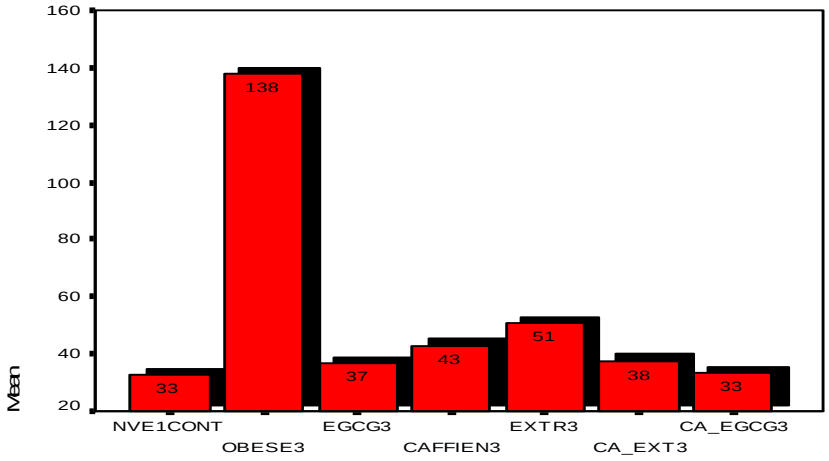

Fig (2): comparison of TNF-α level between different groups and normal group 9weeks of treatment

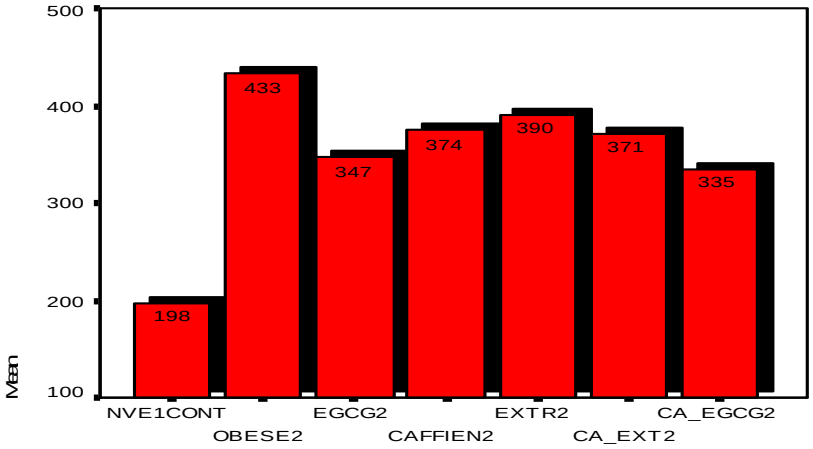

Fig (3): comparison of IL-6 level between different groups and normal group after5 weeks of tratment

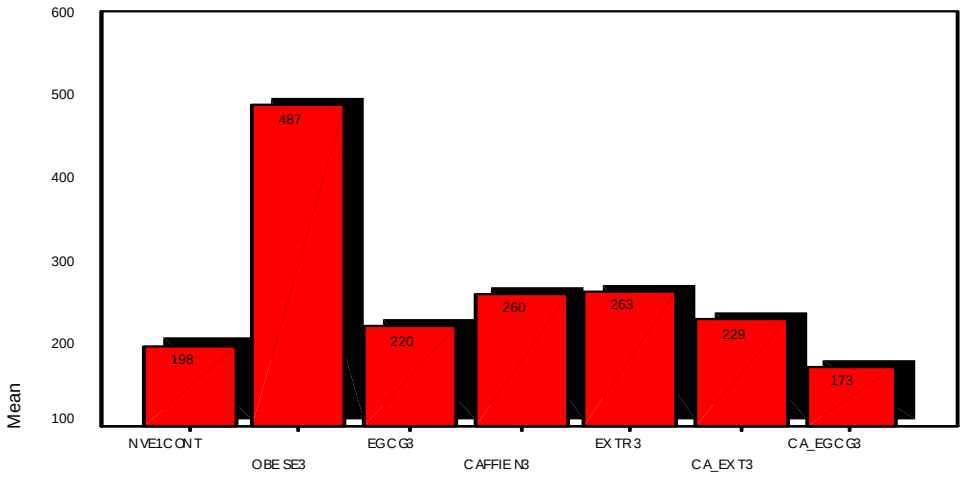

Fig (4): Comparison of IL-6 level between different groups and normal group after 9 weeks of treatment

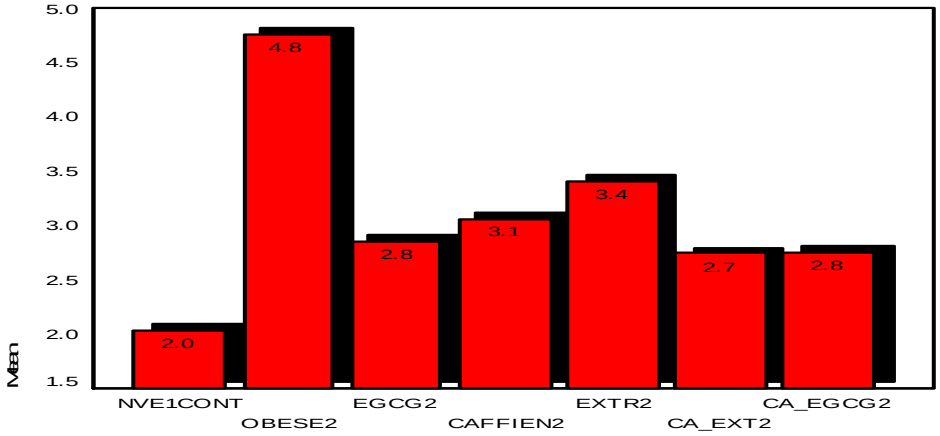

Fig (5): comparison of CRP levels in different groups and normal group 5weeks of treatment

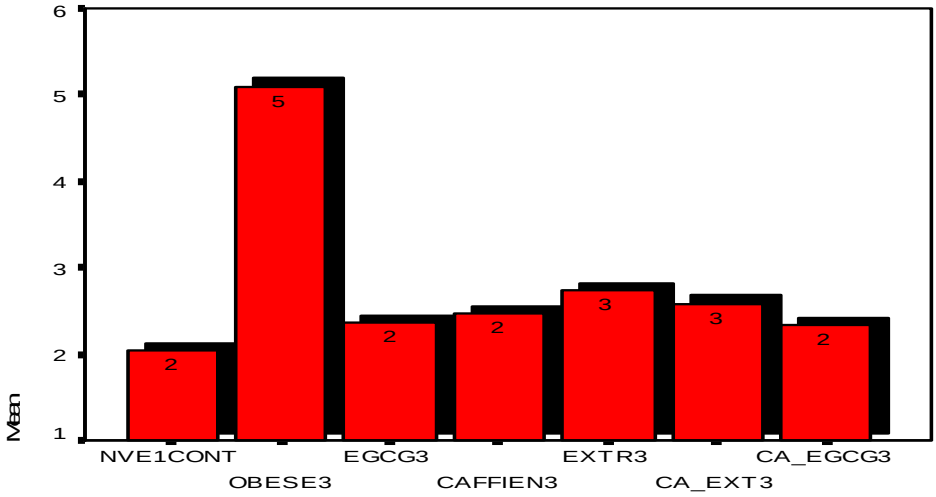

Fig (6): comparison of CRP levels in different groups and normal group 9weeks of treatment

4. Discussion:

Tea is one of the most widely consumed beverages in the world and is second only to water in popularity as beverage. Consumption of tea has been associated with many health benefits including prevention of cancer and heart disease; (shenmin et al., 2011). Theses effects are attributed to the polyphenol compounds in tea. Different mechanisms of action have been proposed for the observed beneficial effects of tea polyphenol.

Inflammatory activity is a characteristic of pathological process in obesity .Adipose tissue is an important source of cytokines and adiposity contributes to pro-inflammatory milieu, (Yu-Wen et al; 2009). It is well known that higher amount of adipocytes leads to misbalance in the immune system causing a state of low grade chronic inflammation leading to marked elevated concentration of proinflammatory cytokines, (Dixit; 2008, Fain; 2006|).

Plasma TNF- α and IL-6 are produced by adipocytes and are elevated in obese subjects .TNF-α also induces IL-6 production which in turn regulates the production of CRP in the liver. Therefore, as TNF-α and IL-6 production is reduced by weight loss, one would expect CRP to reduced as well. There was some evidence that caloric restriction alone damps down the inflammatory response by reducing the DNA binding affinity of NF-kB which modulates the production of cytokines IL-B and TNF-α (Leonie et al., 2002) Adipocytes secretes IL-6 ,one of chief inducers of CRP production by the liver .Approximately ,30% of circulating IL-6 is estimated to be from adipose tissue.(Hye Soon Parka et al., 2005), Proinflammatory cytokines were increased 3-fold for IL-6 and 7-fold for TNF-α in obese animals (Patrick et al., 2010). High level of IL-6 are responsible for the increase in acute-phase proteins seen in obese patients, particularly CRP, via its action on CRP-production cells in the liver (Michael et al., 2008).The elevated CRP concentration can be ascribed to increased expression of IL-6 in adipose tissue and its release into circulation .IL-6 is a pro-inflammatory cytokine that stimulates the production of CRP in the liver (Das, and FAMS., 2001).

Visser et al; (2001), demonstrated that higher BMI is associated with higher CRP concentration in young adults aged 17-39 years, thus confirming a state of low systemic inflammation on overweight and obese persons.(Das, and FAMS., 2001).

Our results showed elevated levels of TNF-α, IL-6 and CRP in obese rats fed high-fat diet when compared with the normal control rats fed normal diet. Which were coinciding with the previous studies. These results were reversed by supplementation the rats with a combination of EGCG with caffeine, EGCG, caffeine, caffeine with green tea, and green tea extract alone. (Chen et al; 2004), found a decrease in lipid peroxidation and inflammation in carbontetrachlorid treated mice which were fed high-fat diet when supplemented with EGCG.

EGCG has been shown to increase NF-E2 related factor-2 (NrF2)nuclear translocation and antioxidant response element (ARE)and up regulate NrF2 protein expression in mouse model .The antioxidant activities are important because membrane peroxidation induces cellular damage and activates NF-kB mediated responses that induces proinflammatory cytokines expression including TNF-α. EGCG has been shown to decrease TNF-α production in vitro by blocking NF-kB activation via the inhibition of IkB kinase, (Chen et al; 2002). EGCG inhibit transcription factor- mediated gene activation such as that via NF-kB and AP-1, (Ahmed et al; 2000). Inhibition of NF-kB and AP-1 mediated gene activation is the central phenomenon that explain the convergence in the antioxidant activity of the green tea catechins and the effect on specific mediator targets NF-kB, which in response to ROs , activates transcription of many proinflammatory and anti-opoptotic /survival gene, (Schoon and Piette; 2000). The ROs –scavenging activity of green tea catechins inhibits NF-kB activation –leading to inhibition of expression of the proinflammatory and survival genes. In addition, EGCG has been shown to directly inhibit proteasome activity, (Nam et al; 2001), leading to accumulation of NF-kB inhibitory protein 1KB, and other pro-apoptotic proteins such as Bax. Inhibition of NF-kB mediated gene activation is also the likely mechanism of inhibition of inducible nitric oxide synthase observed with green tea and EGCG ,which mediates its anti-inflammatory actions, (Singh et al ;2002; and Vadivel et al ; 2009) , showed that EGCG decreased lipopolysaccharide induced TNF-α production in mice and in a macrophage cell line.

Abe et al; (2005), stated that green tea cause down regulation of TNF-α gene expression .The molecular mechanism of anti-inflammatory effects of green tea may partially due to its ability to increase mRNA levels encoding anti-inflammatory factors such as TTP/TISII/ZF P36 and /or ELAVL1/HUA/HUR (Tris tetraprolin Family proteins ,zinc finger protein 36 ,Human antigen R, Embryonic lethal abnormal vision –like 1) and decrease mRNA levels encoding pro-inflammatory factors such as TNF-α and or Cox-2/PTGs 2 (cyclooxygenase-2/prostaglandin endoperoxidase), (Heping et al; 2007). Green tea extract regulate TNF –α gene expression by modulating NF-kB activation

though their inhibition effect on Ikkb kinase activity and as scavenger of free radicals. Treatment of green tea reduced oxLDL-induced production of pro-inflammatory cytokines TNF-α and NF-kB activation in a dose dependent manner (Wahyudi, and Sargowo; 2007). (Ramesh et al; 2009), have shown anti-inflammatory effects of intaperitoneal administration of EGCG in significantly reducing serum CRP levels and hematologic markers of inflammation on rats fed on atherogenic diet versus the untreated group. In same time (Ryua et al; 2005), examined the effect of green tea on inflammation, they stated that the inflammatory markers such as CRP and IL-6 were unchanged after green tea consumption.

Hirofumi; (2011), found that EGCG reduced Toll-like receptor-4 (TLR-4) expression (which induce inflammatory mediators) through 67LR (67Lreceptor). EGCG induced a rapid up regulation of Tollip protein, a negative regulator of TLR –signaling, and this EGCG action was prevented by 67LR silencing or anti-67LR antibody treatment. (Hirofumiand Tatsuo; 2011),

Our results showed that caffeine had an anti-inflammatory effect that appeared through inhibiting TNF-α and subsequently IL-6 and CRP. It enhances the anti-inflammatory effect of EGCG and green tea extract when it was add to them.

Caffeine exerts most if not all of its effect via antagonism of adenosine receptors. Caffeine is also a well –known inhibitor of cyclic adenosine monophosphate-phosphodiesterase (cAMP-PDE). As adenosine mediates many anti-inflammatory effects, antagonism of adenosine receptors by caffeine would be expected to inhibit the anti-inflammatory effects of endogenous adenosine, and possibly elect a pro-inflammatory effect. However, as the evidence clearly indicates that caffeine is generally anti-inflammatory, attention has been directed towered other possible mechanisms of action, (Horrigun et al; 2004).

Caffeine increased the concentration of intracellular cAMP and subsequent activation of protein kinase A (PKA) .cAMP is very potent immunomodulatory, exerting generally supperssine effect on the functions of inflammatory and immuno competent cells . Activation of cAMP/PKA pathway inhibits lipopolysaccharide (LPS) stimulated production of proinflammatory cytokines such as TNF-α, (L.H Horrigan et al; 2006). Furthermore the suppressive effect of caffeine on TNF-α production was blocked by the high specific cAMP antagonist Rp-8-Br-cAMPS. Similarly Rosental et al ;(1992) demonstrated that HA1000, an inhibitor of cAMP and cyclic guanosine monophosphate (cGMP) dependant protein kinases, blocked caffeine –mediated inhibition of sheep red blood cells specific antibody responses by murine spleen cells in vivo. These data suggest that the immune-modulatory actions of caffeine are mediated by inhibition of cAMP-PDE and resultant increase in intracellular cAMP concentration. Furthermore approximately 80% of caffeine is converted to parxanthine which is one of phosphodiesterase (PDE) inhibitors .Xanthines have extensive effects on immune function inducing suppression of pro-inflammatory cytokine release (including TNF-α, IL-2 and interferon-γ).Stimulating of anti-inflammatory cytokine (IL-10), and suppression of neytrophil and eosinophil chemotaxis and superoxide generation, (L.A.Horrigan et al; 2006).

Another pathway for immune modulatory effect of caffeine is through inhibition of poly (ADP-ribose) polymerase-1 (PARP-1) a nuclear enzyme present in eukaryotes, PARP-1 is reported to play a role in NF-kB and AP-1 mediated production of pro-inflammatory cytokines, (Liesbeth et al; 2006).

We conclude that the addition of caffeine to EGCG or green tea extract enhance their anti-inflammatory effects on the low grade inflammation state a combined obesity by decreasing the levels of TNF-α,IL-6 and CRP. As inflammation known to be one of the major causes of CVD and Diabetes, this result could be beneficial in decreasing the side effects of obesity which cause low grade chronic inflammation.

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Effects of Prenatal Phenytoin Toxicity on the Expression of Glial Fibrillary Acidic Protein (GFAP) in the Developing Rat Cerebellum

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Abstract The Cerebellum is a highly organized structure and its postnatal development was characterized by cell proliferation, migration and differentiation. Phenytoin is a primary antiepileptic drug used for all types of epilepsy except absence seizures. Even with the newer antiepileptic drugs, phenytoin continues to serve as a reference point and many epileptic women received phenytoin during pregnancy. The mechanism of teratogenicity by phenytoin is still under investigation. So, the present study was designed to clarify the effect of prenatal phenytoin on the cerebellar development in rat offspring using the immunohistochemical distribution of glial marker. The pregnant rats were received phenytoin 35mg/ kg body weight once a day from gestational days E5 to E20. In H and E stained sections, the Purkinje cells in the treated group (PD7) had poor and immature arbors and partially showed an irregular arrangement. There is dispersal of the internal granular cell layer and the white matter with the presence of vacuolations, dilated capillaries and extracellular oedema. The marker of radial glia, glial fibrillary acidic protein (GFAP) has been used to describe phenytoin induced alteration in the morphology and reactivity of Bergman glial cells and their fibres that are the guide substrate of granule cells. The feature of these fibres gives information on the proper granule cell migration. GFAP positive immunoreactivity was first detected at postnatal day one (PD1). Thin glial positive fibers had a regular feature running in parallel in the molecular layer and in the external granular layer of controls at postnatal day seven (PD7). In contrast, in the treated rats, the glial fibers appeared twisted, thickened with an uneven course and strongly labeled end feet. GFAP immunoreactivity in the white matter astrocytes was highly detected in both the control and the treated PD7. From the previous findings, it could be concluded that phenytoin has degenerative changes on the cerebellar development. These changes can lead to extensive neurological poor health effects later in life.
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Keywords: Cerebellum; Phenytoin; Purkinje cells; Bergman glial fibers; Astrocytes; GFAP

Abbreviations: E5, E20, embryonic day 5, 20; GFAP, glial fibrillary acidic protein; PD1, PD7; postnatal day 1, 7

1. Introduction:

The cerebellum is a highly organized structure in which the Purkinje cells are the sole output of the cerebellar cortex **(Altman, 1969; 1972a-c).** Significantly, the cerebellum is one of the first structures to differentiate however; it achieves its mature configuration after birth **(Bouet et al., 2005).** Therefore, the cerebellum is especially vulnerable to developmental irregularities **(Kern et al., 2006).**

During postnatal development of rat cerebellum, the external granule cell layer represents the matrix area in which two discrete zones can be clearly recognized, the proliferative and the premigratory zones **(Altman, 1972a-c).** The postmitotic cells of external granular layer migrate to their final destination in the internal granule cell layer **(Rakic, 1971 and Altman, 1972a).** Definitely, the precise positioning of the cells is important for the final cytoarchitecture and in particular for the pattern of synaptic connections in laminated brain regions such as cerebellum **(Rakic et al., 1994).**

Maternal treatment with antiepileptic drugs can lead to cognitive dysfunction in later life of offspring and probably increasing the risk of central nervous system disorder. Several investigations demonstrated that the children of epileptic women who received antiepileptic drugs during pregnancy might have mild mental retardation **(Scolnik et al., 1994).** Phenytoin is a primary anti-epileptic drug used for all types of epilepsy except absence seizures **(McNamara, 2001).** Significantly, many epileptic women received phenytoin during pregnancy **(Kelly et al., 1984; Lander and Eadie, 1991).** There is some evidence that phenytoin causes brain damage. Gestational administration of phenytoin in rats can reduce whole brain weight **(Tachibana et al., 1996),** delay maturation of reflexes **(Dam, 1972),** and change postnatal behaviors such as increased spontaneous locomotion as well as learning impairments

(Vorhees, 1987; Adams et al., 1990 and Pizzi and Jersey, 1992).

The purpose of this study is to clarify the effect of prenatal phenytoin administration on the cerebellar development in rat offspring. The present work was carried out in rat offspring on PD1 and PD7 born from mothers treated by phenytoin during the gestation period. In this study, the immuno-histochemical marker of glial polypeptides was used. A marker of radial glia, glial fibrillary acidic protein (GFAP) has been analysed here to describe phenytoin induced alteration in the morphology and reactivity of radial glial fibers. GFAP was discovered by Eng et al. in 1971 during an analysis of multiple sclerosis plaques **(Eng et al., 1971).** GFAP was found to be an intermediate filament protein expressed almost exclusively in astrocytes, leading to its adoption as an astrocytic marker for both clinical and basic studies **(Eng and Ghirnikar, 1994; McLendon and Bigner, 1994).**

2. Materials and Methods:

Experimental animals

40 adult albino rats (33 female and 7 male) were used. The rats were housed in animal care centre of faculty of Medicine for Girls, AL-Azhar University. They were kept under similar conditions of wire-floored cages, adequate ventilation and temperature with free access to water and food.

Experimental treatment & groups

The rats were mated overnight, and the day on which a vaginal plug was found was selected as gestational day 0. The pregnant rats were housed separately in a plastic cage. The pregnant rats (270-300g) were treated with phenytoin orally. Phenytoin (Phenytin) produced by the (The Nile for Pharmaceuticals and Chemical Industries- CO. Cairo-A.R.E.). Phenytin was in the form of tablets, which were freshly dissolved in distilled water and given orally by gastric tube to the rats. Rats were received phenytoin at a dose of 35mg/ kg body weight **(Ohmori et al., 1999)** once a day from gestational days E5 to E20. The day of birth was designated as postnatal day 0. In the postnatal period, the male rat's offspring cerebellum of both the treated and the control groups were selected at the following ages: postnatal day one (PD1) and postnatal day seven (PD7). 40 male rats' offspring were used. In the postnatal period, rat pups were grouped as follows:

Group 1: Control male rat pups (PD1) born from the control pregnant rats.

Group 2: Treated male rat pups (PD1) born from the pregnant rats treated by phenytoin.

Group 3: Control male rat pups (PD7) born from the control pregnant rats.

Group 4: Treated male rat pups (PD7) born from the pregnant rats treated by phenytoin.

Histopathological analysis

Each offspring Rat was anaesthetized with diethyl ether. The brains were removed and immersed in Bouin's fixative for 24 hours. The brains were then dehydrated in ascending grades of ethanol, embedded in paraffin and serially sectioned in median sagittal plane at a thickness of 4-5μm. Sections were stained with Haematoxylin and Eosin for histological examination according to the method of **(Drury and Wallington, 1980).**

GFAP immunohistochemistry

The tissue blocks were cut at 4μm thickness, deparaffinized and rehydrated to buffer (water). The sections were incubated with optimally diluted GFAP, mouse monoclonal antibody **(Dako N-series Ready- to use primary antibody)**. The immunostaining was amplified and completed by Hoarseradish Peroxidase complex **(Dako REAL™ EnVision ™ / HRP, Mouse ENV).** Sections were developed and visualized using 3,3diaminobenzidine **(Dako REAL ™ DAB+ Chromogen).** The substrate system produces a crisp brown end product at the site of the target antigen. Sections were counterstained with or without haematoxylin. The sections were then dehydrated in alcohol, cleared in xylene and coverslipped with Permount. The Sections were examined under light microscopy.

3. Results:

Group 1: Control postnatal day one (PD1) rats

In H& E stained sections, the cerebellum was present in the dorsal part of the hindbrain. The cerebellum consisted of normal lobules and fissures (Fig.1A). The cerebellar cortex is built up of, from outside inwards, the external granular layer, the molecular layer and the internal granular layer. The external granular layer consisted of 3-5 cell layers. The cells of the external granular were oval or rounded in shape with darkly stained nuclei. They are densely aggregated superficial zone and less packed deep zone. The molecular layer is a pale narrow zone with loose aggregation of cells. The Purkinje cells could barely be seen. The Purkinje cells could be observed mixed together among cells of the internal granular layer or as an indistinct layer superficial to the cells of the internal granular layer. The internal granular layer is relatively a thick layer with deeply stained cells of different shape and size. The white matter is observed beneath the internal granular layer and it is stuffed with a great number of less packed fusiform or oval cells (Fig.1B).

GFAP immunoreactivity: GFAP positive immunostaining was detected in the external granular layer and in the molecular layer (Fig.1 C& D).

Group 2: Treated postnatal day one (PD1) rats

In H& E stained sections, the cerebella revealed slightly decrease in its size as compared with the control. The fissures became shallow and poorly developed in comparison with the control one. There is obvious decreased in the depth of the fissures. Moreover, there is slightly delayed appearance of some cerebellar fissures (Fig.2A). The cerebellar cortex showed that there is slightly dispersion of cerebellar cortical layers with the presence of cavitations and extracellular oedema. The external granular layer was thin in comparison to the control. The cells of the external granular layer were small rounded or oval in shape with deeply stained nuclei. The molecular layer could hardly be recognized. It appeared very thin and weakly developed as compared with the control. The Purkinje cell layers could not be seen similar to the control one. The internal granular layer consisted of small rounded or oval cells with deeply stained nuclei. The cells of the internal granular layer were dissociated from each other and some of them had vacuolated cytoplasm (Fig.2B).

GFAP immunoreactivity: GFAP positive immunoreactivity was observed in the external granular layer and in the molecular layer (Fig. 2 C&D).

Group 3: Control postnatal day seven (PD7) rats

In H& E stained sections, the cerebellar cortex became more developed. The cerebellum is obviously increased in size and its fissures are increased in depth as compared with the previous control age. In addition, the development and differentiation of the new fissures were also observed (Fig.3 A). The cerebellar cortex is built up, from outside inside, the external granular layer, the molecular layer, the Purkinje cell layer and the white matter. The external granular layer showed marked increase in thickness in comparison to the PD1. The molecular layer showed marked development and apparantly increased in thickness (Fig.3 B, C& D). The Purkinje cell layer is arranged in one row. The Purkinje cells were typically oval or fusiform in shape with their long axes vertical to the surface. The elongated dendrites of Purkinje cells in the molecular layer are visible. Dendritic arbors are also clearly observed. Most of the Purkinje cells are aligned in a regular pattern (Fig.3 B, C& D). The internal granular layer is thicker than that of the control of the previous age. The cells of the internal granular layer are rounded or oval with deeply stained nuclei. The white matter is well developed and clearly detected underneath the internal granular layer (Fig.3 B& C).

GFAP immunoreactivity: the GFAP positive fibers were thin and had a regular feature running in parallel in the molecular and in the external granular layers (Fig.4 A-D). The GFAP is highly expressed in the astrocytes in the white matter (Fig. 4 E-F).

Group 4: Treated postnatal day seven (PD7) rats

In H& E stained sections the cerebellum was slightly less developed (Fig.5A) in comparison to the control of the same age (Fig.3.A). The cerebellar cortex consists of from outside inwards, the external granular layer, the molecular layer, the Purkinje cell layer and the internal granular layer. Significantly, extensive hemorrhage along the cerebellar cortical layers was also observed (Fig.5B). The external granular layer was to some extent less developed than that of the control group. In addition, the cells in the external granular layer appeared small in size with deeply stained or pyknotic nuclei. Apparently, the width of molecular layer (Fig.5B, C&D) was relatively decreased in comparison to the control. The molecular layer cells were loosely packed. The Purkinje cells were disarranged in one row with less stained cytoplasm and less diverse nuclei. In addition, focal loss of Purkinje cells is observable (Fig. 5B, C&D). Dendritic arbors appeared poorly developed and immature. Some of Purkinje cells are aligned irregularly (Fig.5D). There is dispersion of the internal granular cell layer and the white matter with the presence of vacuolations, dilated capillaries and extracellular oedema (Fig.5 C& D).

GFAP immunoreactivity: Importantly, the glial fibers emerged twisted with an uneven course and sometimes they navigated the molecular layer at different angles. Sometimes, the fibers appeared disrupted. Then, few GFAP positive fibers (Fig. 6 A-D) frequently had thickened and intensely stained end-feet in the pial surface of the external granular layer (Fig.6 C). The GFAP is highly detected in astrocytes in the cerebellar white matter (Fig.6.E-F).

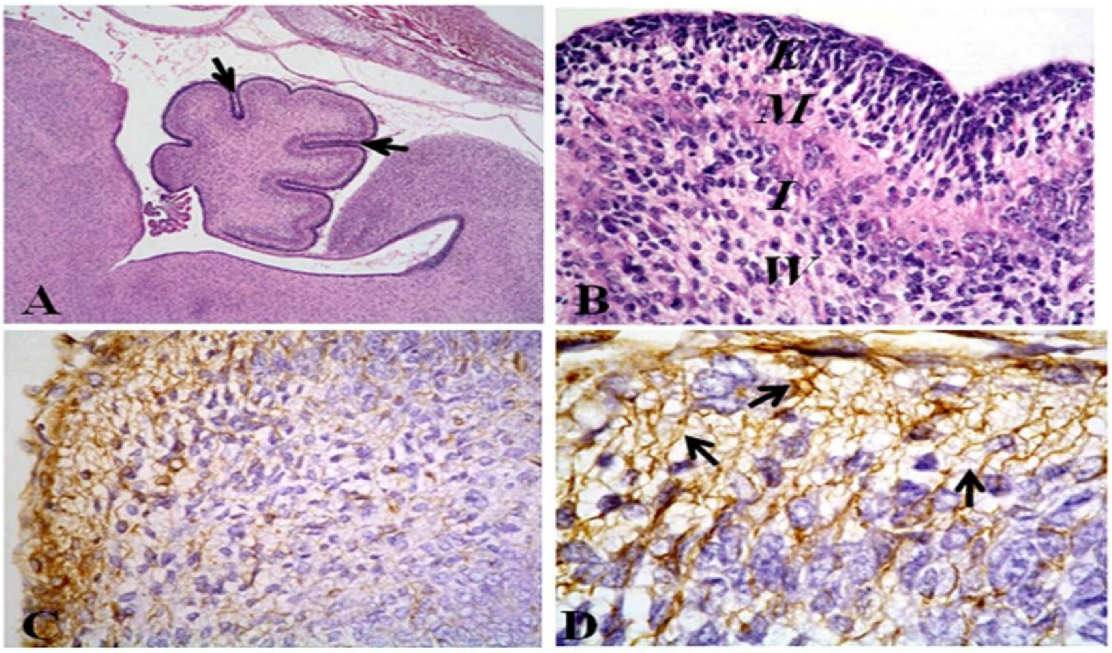

Fig.1. Photomicrographs of the median sagittal sections of cerebellum of the control postnatal day one (PD1) rats (A- D). (Group 1)

(A) Showing the normal architecture of the cerebellum with many fissures (arrows) and lobules.

(H and E x40)

(B) Showing the layers of the cerebellar cortex namely; the external granular layer (E), the molecular layer (M) and the internal granular layer (I). The white matter (W) is observed beneath the internal granular layer.

(H and E x400)

(C) Showing distribution of GFAP positive immunoreactivity in the external granular layer and in the molecular layer.

(x400)

(D) High magnification of C to show the intense GFAP staining pattern in the glial positive fibers in the external granular layer and in the molecular layer (arrows). (x1000)

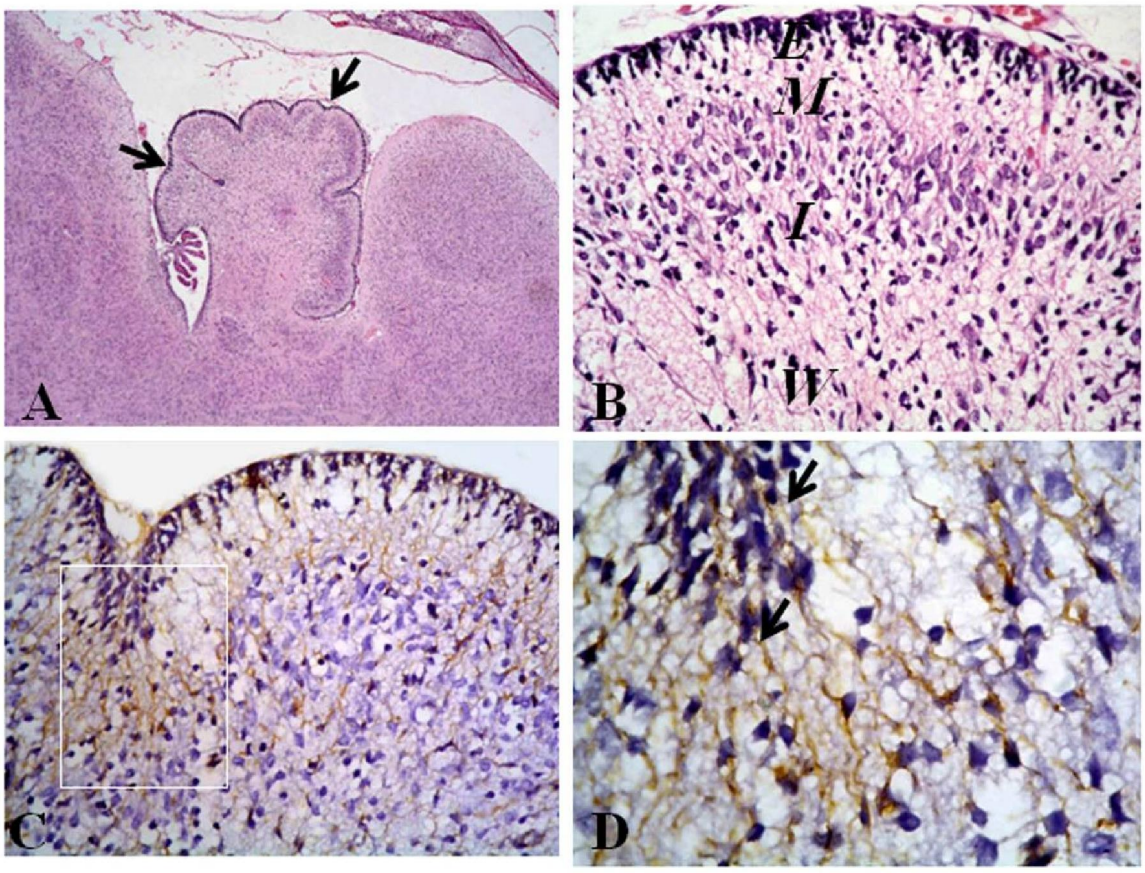

Fig.2. Photomicrographs of the median sagittal sections of cerebellum of postnatal day one (PD1) rats of treated mothers (A- D). (Group 2)

(A) Showing that the cerebellum was less developed. Marked reduction of the depth of the fissures (arrows) is observed.

(H and E x 40)

(B) Showing the poorly developed of the external granular layer (E). The molecular layer (M) appeared as a very thin narrow zone and the internal granular layer (I) are of low density with small size of cells and some of them have vacuolated cytoplasm.

(H and E x400)

(C) Showing distribution of GFAP immunoreactivity in the external granular layer and in the molecular layer.

(x400)

(D) The figure D are the high magnification of the white frame in (C) to show the positive brown immunostaining pattern of GFAP in the external granular and molecular layers (arrows).

(x1000)

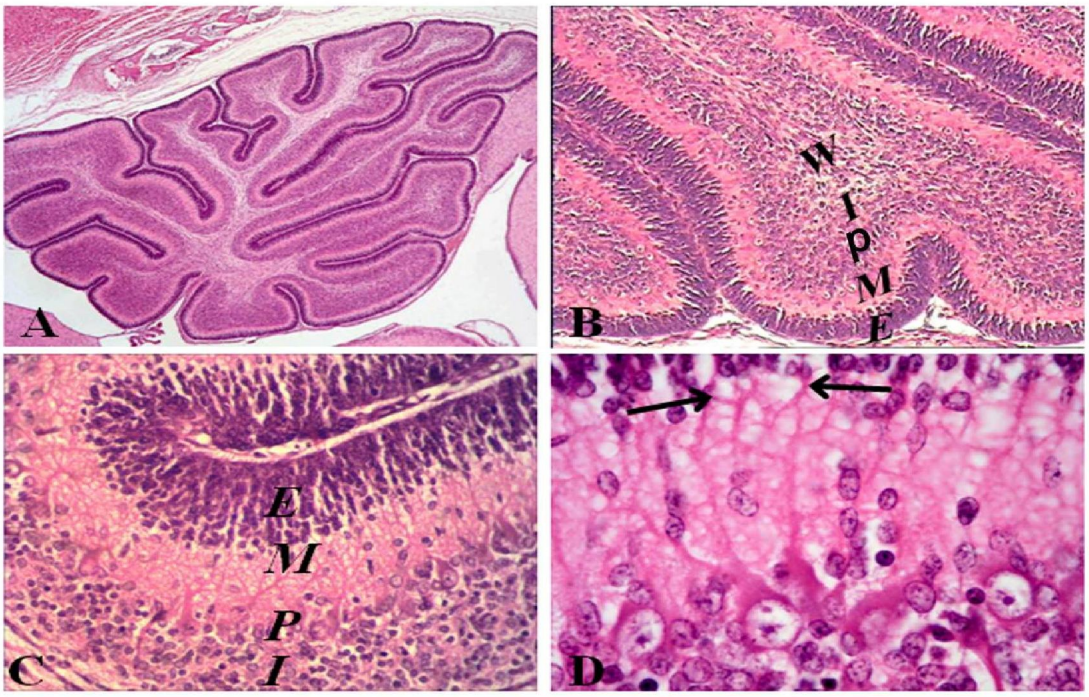

Fig.3. Photomicrographs of the median sagittal sections of cerebellum of the control postnatal day seven (PD7) rats (A- D). (Group 3)

(A) Showing that the cerebellum is markedly increased in size. The development and the differentiation of the new fissures are clearly observed.

(H and E x40)

(B) Showing the well developed of the cerebellar cortical layers namely from outside inwards, the external granular layer (E), the molecular layer (M), the Purkinje cell layer (P) and the internal granular layer. The white matter (W) is clearly distinguished.

(H and E x100)

(C) Showing the well demarcated of the cerebellar cortical layers.

(H and E x400)

(D) Showing the Purkinje cells are well developed and stuffed in one raw. The elongated dendrites of Purkinje cells in the molecular layer are evident. Importantly, dendritic arbors are also clearly observed (arrows). In addition, most of the Purkinje cells are aligned in a regular pattern.

(H and E x1000)

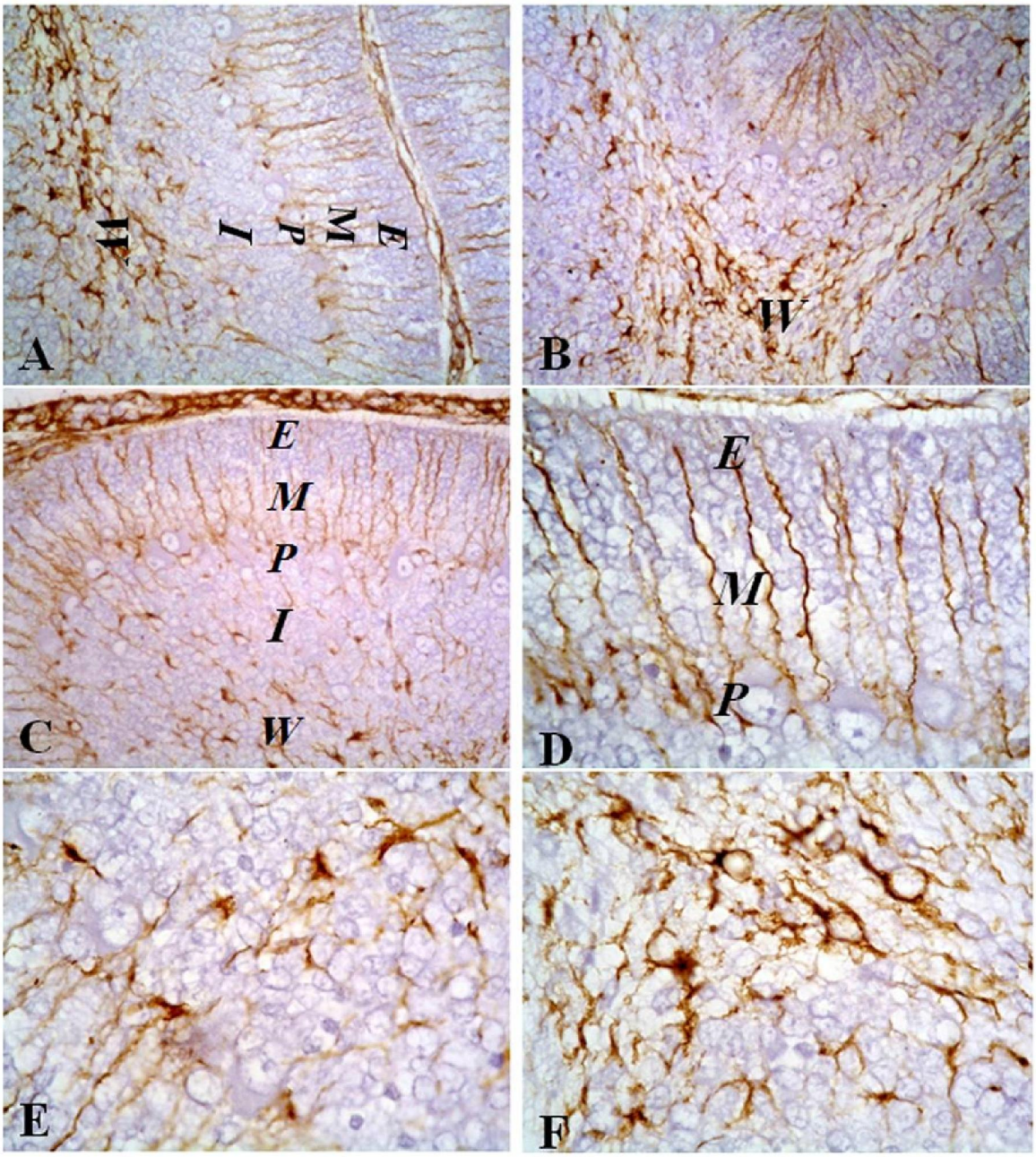

Fig.4. Photomicrographs of the median sagittal sections of cerebellum of the control postnatal day seven (PD7) rats (A- F). (Group 3)

(A-D) Showing the GFAP positive radial glial fibers appear thin and regular in the external granular layer (E) and in the molecular layer (M).

(E-F) The strong GFAP positive immunostaining in the astrocytes in white matter is observed.

(A-B-C x 400) (D- E-F x1000)

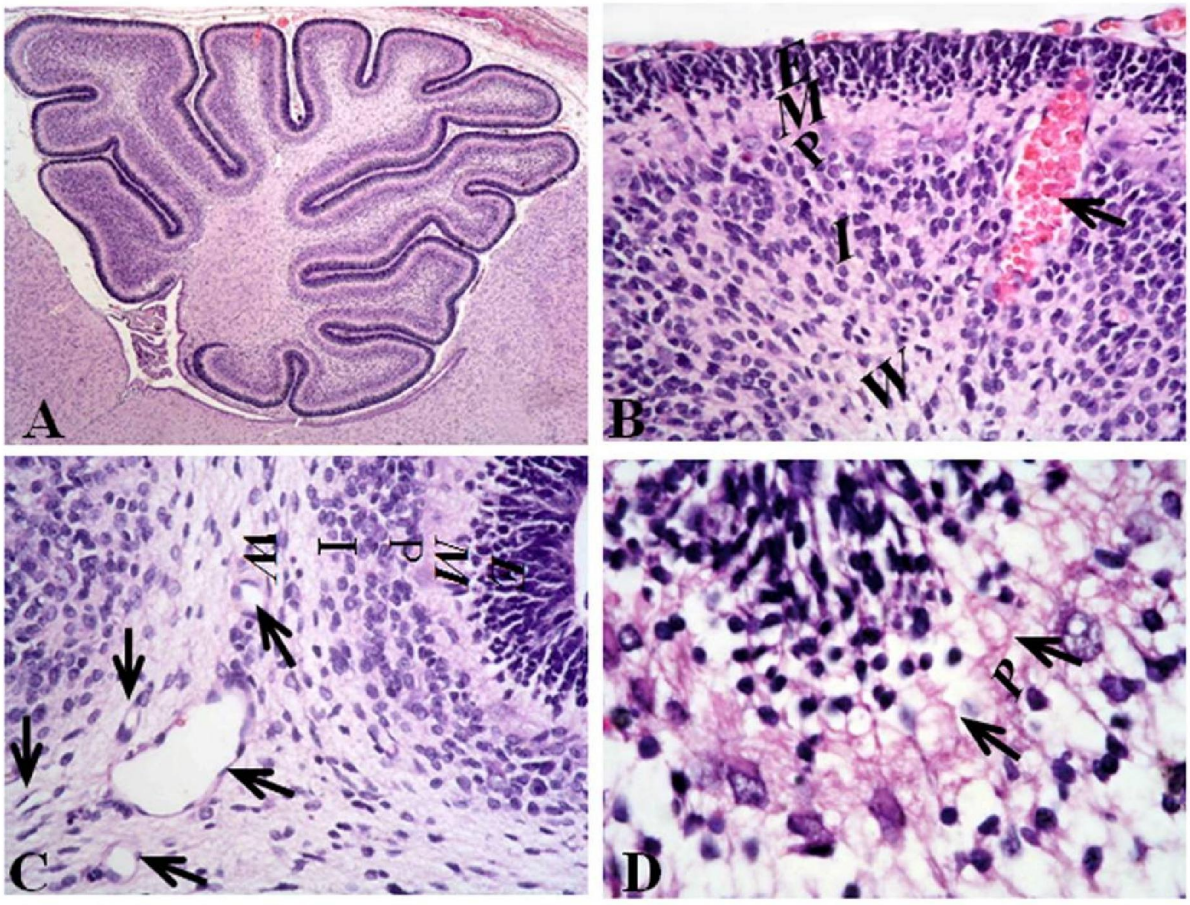

Fig. 5. Photomicrographs of the median sagittal sections of cerebellum of the postnatal day seven (PD7) rats of treated mothers (A- D). (Group 4)
(A) Showing that the size of the cerebellum is slightly decreased.
(H and E x40)
(B) Showing the external granular layer (E) and the molecular layer (M) are poorly developed. Diffusion of the internal granular layer (I) and extensive hemorrhage (arrow) along the cerebellar cortical layers are clearly noticed.
(H and E x400)
(C) Showing dispersion of the white matter with the presence of dilated capillary of blood vessels and cavitations (arrows).
(H and E x400)
(D) Showing moderately focal loss (P) of Purkinje cells. Significantly, the Purkinje cells had poor and immature arbors (arrows) and partially showed an uneven arrangement.
(H and E x1000)

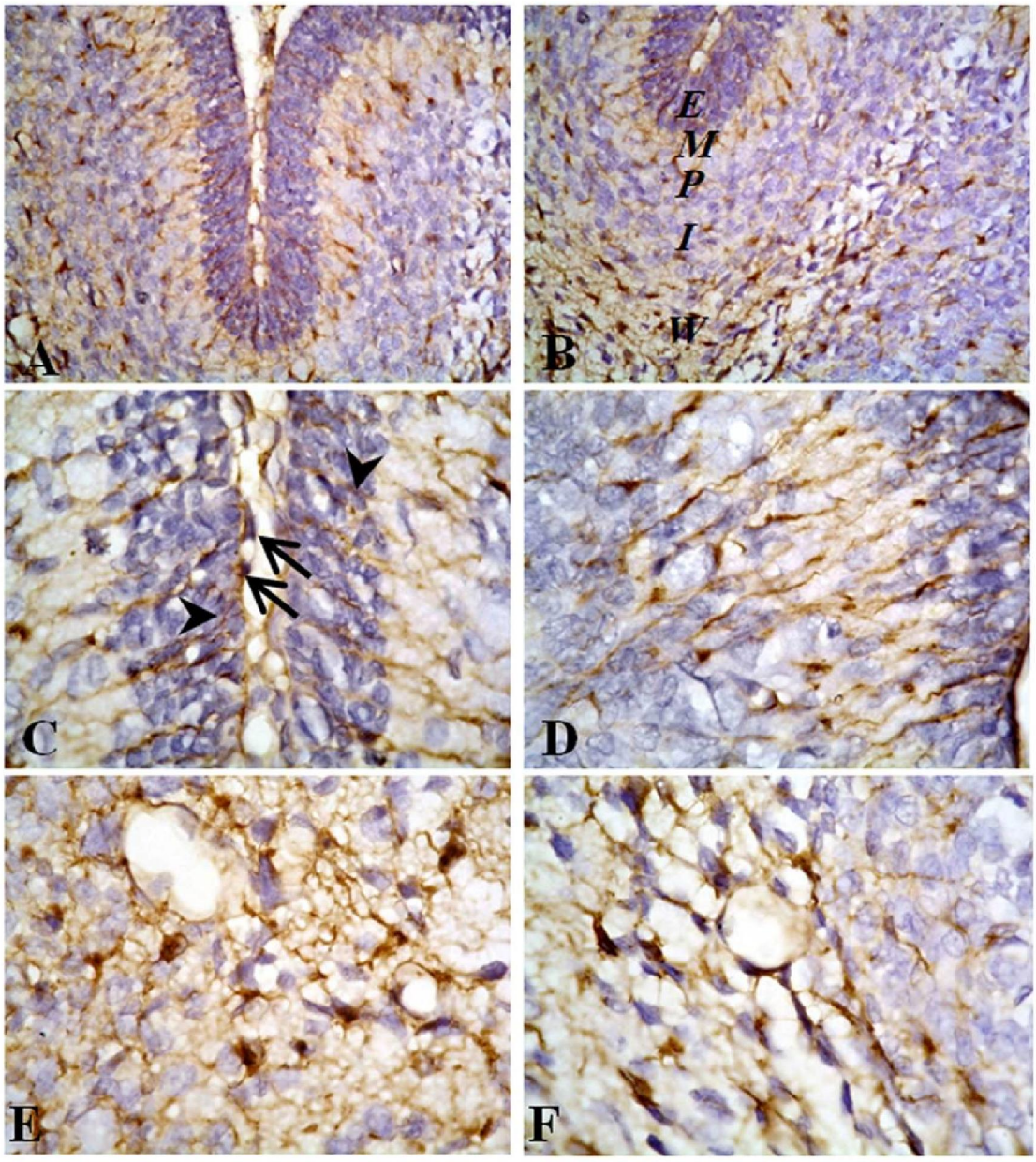

Fig.6. Photomicrographs of the median sagittal sections of cerebellum of the postnatal day seven (PD7) rats of treated mothers (A- F). (Group 4)

(A-D) Showing the GFAP positive radial glial fibers are twisted, thickened, interrupted (arrowheads), and loose their parallel course. Moreover in (C) some of these fibers have intensely stained thickening (end- feet) at the pial surface of the external granular layer (arrows).

(E-F) Showing the GFAP positive imunostaining in the astrocytes in white matter are noticed.

(A- B x 400) (C- D-E -F x1000)

4. Discussion

Phenytoin is one of the most effective antiepileptic drugs that it is widely used in most forms of epilepsy except absence seizures **(McNamara, 2001).** The neurotoxicity of phenytoin has been investigated. Clinico-epidemiologic studies suggested an approximated two to three-fold increase in malformations in neonates exposed to phenytoin during the prenatal period **(Janz, 1982 and Kaneko et al., 1988).**

The mechanism of teratogenicity by phenytoin is still under analysis. The present study was carried out, in a trial to elucidate the cause of cerebellar injury with special reference to its effect with both histological and immunohistochemical studies following maternal administration of phenytoin during the gestation period. The current study evidenced that administration of phenytoin to the pregnant rats extensively influenced the cerebellum of offspring on PD1 and on PD7 groups. These results could be attributed to the findings of **(Vorhees et al., 1988; Adams et al., 1990; Pizzi and Jersey, 1992).**

In the present study, phenytoin diminished the rate of growth of the cerebellum. The cerebella of treated groups at PD1 and PD7 were less developed as compared with the control. These results were in harmony with **Gadisseaux et al. (1984)** who stated a novel cases of ponto-neocerebellar hypoplasia in children born to mothers treated with phenytoin during pregnancy. Moreover, **Squier et al. (1990)** have demonstrated no Purkinje cells in any part of the cerebellar hemispheres and cerebellar hypoplasia accompanied by extensive gliosis. Furthermore, total brain weight was lower following neonatal administration of phenytoin in mice than the control group and the reduction of brain weight was more remarkable in comparison to body weight **(Ogura et al., 2002).** The same result was obtained in the chick cerebellum. **Allam et al. (1987)** had explained that phenytoin (epanutin) produced reduction of the activity of the cells in the external granular layer leading to shrinking of the size of the cerebellum.

The present work showed that the cerebellar fissures became shallow and poorly developed in the treated group on PD1 than that of the control group. Significantly an apparent reduction in the depth of the fissures was also observed. Indeed, cerebellar malformations have been reported following intrauterine exposure to anticonvulsants drugs including phenytoin **(Gadisseaux et al. 1984; Squier et al. 1990)**.

The present study showed that the external granular layer of the control group on PD7 is more developed than that of the control group on PD1. These observations receive a marked support from the reports issued by **Noor-El-Din et al. (1977)** who reported that the thickness of the external granular layer increased gradually to reach its maximum at the age of 7days.

In this study, the external granular layer apparently is thinner of the treated group on PD1 than that of the control group. In addition, the cells of the external granular layer are deeply stained. The possible explanation of the reduction of the thickness of the external granular layer principally as a result of the high number of apoptotic cells in the cerebellum. Two anticonvulsants, carbamazepine and phenytoin have been shown to induce apoptosis of cerebellar granule cells at doses higher than their therapeutic levels **(Yan et al., 1995).** The changes of the external granular layer in the previous literatures were outstanding compared with those of other cerebellar cells. **Ohmori et al. (1992)** reported that, oral administration of phenytoin in the neonatal period induces neurotoxic effects on the developing mouse cerebellum**.** The cells of the external granular layer were pyknotic cells and the external granular layer was thicker than that of the control. These changes were observed in high (50mg/Kg) or low doses (25-35mg/Kg) of phenytoin. These results suggested that phenytoin induces cell death of the external granule cells and inhibits proliferation and migration of granule cells in newborn mouse cerebella **(Ohmori et al., 1992; 1997 and 1999).** In addition**,** neonatal administration of phenytoin (35 mg/Kg) daily during postnatal days 5-14 revealed that the layer of the mature granule cells in the dentate gyrus was thinner in the phenytoin treated mice **(Ogura et al., 2002).** Their results demonstrated that neonatal administration of phenytoin interferes with the development of granule cells in the hippocampus and the cerebellum and causes spatial learning deficits in later life. The same results were obtained in the chick cerebellum. Previously, **Allam et al. (1987)** reported that phenytoin (epanutin) inhibited the migration of the cells of the external granular layer. Definitely, agents acting in the perinatal or early postnatal periods are thus liable to interfere with the development of cerebellar granule cells and other cerebellar cells may be affected as a result of loss granule cells **(Altman et al., 1969; Ohmori et al., 1999).** Moreover, the relationship among proliferation, death and migration in the development of cerebellar lobules was largely supported by findings obtained after the cisplatin treatment. Significantly, evident changes of the whole external granular layer were found on postnatal days 11, one day after cisplatin treatment when reduction of the thickness of this layer was found in treated rats, mainly in consequence of the high number of

apoptotic cells in all the cerebellar lobules **(Pisu et al., 2005).**

The present study showed that the molecular layer of the control group is thin on PD1. The molecular layer of the control group is markedly developed and increased in thickness on PD7. The same results were obtained in the rabbit cerebellum. In the rabbit, the molecular layer was observed as a very thin zone on the 19th prenatal days and gradually increased in thickness between 1st and 5th postnatal days **(Noor- El-Din et al., 1985).**

The present study suggested the retardation of the growth of the molecular layer in the treated rats in both PD1 and PD7 as compared to the control. These findings were in agreement with **(Ohmori et al., 1999)** who stated that the molecular layer in the vermis in the treated group was thinner than that of the control group on postnatal days 14 in mouse cerebellum. Similarly, in chick cerebellum, phenytoin reduced the thickness of the molecular layer **(Allam et al., 1987)**.

In the present work, the most impressive alterations in the cerebellum of treated rats on PD7 were delayed the maturation of Purkinje cells. These changes were in the form of immature dendritic development and partially an irregular arrangement of Purkinje cells. These results are in accordance with **Blank et al. (1982)** who applied phenytoin to the developing neonatal mouse cerebellar cultures. Their observations revealed that phenytoin induce Purkinje cell degeneration. Similar changes of the immature dendritic development of the Purkinje cells following phenytoin administration to the newborn mouse once a day during postnatal days 2-4 was also reported **(Ohmori et al., 1999)**. The possible interpretation of the result of the deleterious changes of the Purkinje cells in the present study could be attributed to the toxicity effects of phenytoin. This interpretation received a good support by **Ohmori et al. (1999)** in which they explained that delayed migration of granule cells to the internal granule layer may deteriorate the synaptic connection of parallel fibers with dendrites of Purkinje cells; or phenytoin may directly damage Purkinje cells and their dendritic extensions. Indeed, altered migration of granule cells influences the growth of Purkinje cell dendrites because the granule cells represent trophic factors for Purkinje cell differentiation **(Altman, 1982).** The alterations in the growth and remodeling of Purkinje cell dendrites are an apparent proof of brain plasticity as revealed during postnatal histogenesis after X –ray irradiation or treatment with cytotoxic substances **(Avella et al., 2006; Li et al., 2006).**

Although several literatures of toxicities in animals and side effects in human induced by phenytoin have been published, however, there is not clear evidence to describe phenytoin induced changes in the morphology of Bergmann radial glial fibers and the astrocytes in the white matter. In this concern, the immunoreactivity of the astrocytic marker (GFAP) is evaluated. GFAP is believed to play an important role in the long-term maintenance of brain cytoarchitecture, proper functioning of the blood brain barrier and modulation of neuronal functions **(Liedtke et al., 1996; Shibuki et al., 1996 and Penky et al., 1998).**

The immunohistochemical findings reported in the present study offer significant evidence that GFAP is highly expressed in the cerebellum in all developmental ages of the control and the treated rats. These results coincide with those of **(Pisu et al., 2005**; **Cerri et al., 2010).**

The immunohistochemical results reported in this study provide significant proof that phenytoin induces neurotoxic damage. At PD7 of the treated group, the GFAP- positive radial glial fibers in the external granular layer were interrupted and in the molecular layer, the radial glial fibers were asymmetrical and distorted in comparison with the typical parallel running features of normal fibres at the same age of the control group. These results are in agreement with **Pisu et al. (2005)** who applied a single injection of cisplatin to the developing rat cerebellum. GFAP immuno-fluorescence showed that cisplatin altered the pattern of radial glia in the neocerebellar lobules VI- VIII; in these lobules fibers were lacking or thick and intensely labeled, also in the end feet. Furthermore, injured radial glia fibers were persisted twenty days after cisplatin treatment possibly leading to the presence of ectopic granule cells in the molecular layer **(Pisu et al., 2004)**.

Alterations of Bergmann glia processes was reported two days after a single injection of cytotoxic agent, methylazoxymethanol (MAM) which produces extensive cell death in the external granular layer and over-expression of GFAP in the distal half of Bergman fibers, including the end feet at the pial surface **(Lafarga et al., 1998).** These results have been explained as a reactive response, in which changes in the interactions between Bergmann glia and granule cell precursors have a role **(Lafarga et al., 1998).**

Faults of Bergmann glia from loss to relatively slight alterations in their localization or in the organization, length and end feet contacts of their processes are reported to affect the cerebellar architecture **(Delaney et al., 1996; Kaartinen et al., 2001).**

Astrocytes are the main cell types that principally express GFAP, as these cells are known to play a critical role in neuromodulation and axon

guidance control during development. In addition, these cells are essential in homeostasis preservation **(Ridet et al., 1997).** In the present research, the expression of GFAP immunostaining in the cerebellar white matter was observed in the control and in the treated PD7. As previously reported, prenatal exposure to carbon monoxide, there is an upregulation of GFAP astroglia in the granular layer and in the Bergman glial cells located in the Purkinje cell and molecular layer **(Yamada and Watanabe, 2002)**. Moreover, exposure to nicotine leads to persistently elevated expression of GFAP in the cerebellar white matter rat offspring on PD60 **(Abdel- Rahman et al., 2004)**.

5. Conclusion

In the present work the subsequent reduction of the thickness of the external granular layer, the altered expression of GFAP and the deformed morphology in the radial glial system could early affect the differentiation of Purkinje cells arbour. Changes in this relationship after the phenytoin treatment may result in the disturbed cerebellar cytoarchitecture. These changes can lead to extensive neurological poor health effects later in life.

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Stability Measurement Of Immediate Dental Implants During Healing Process Using Resonance Frequency Analysis

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Abstract: Primary implant stability has been identified as a prerequisite to achieve osseointegration. Recently, Resonance Frequency Analysis (RFA) has been introduced to provide an objective measurement of implant primary stability and implant stability over the healing period. It was hypothesized that determination of a primary stability threshold, provided in terms of a defined Implant Stability Quotient (ISQ) value, might be relevant to predict the osseointegration of a given implant. The purpose of the current study was directed to evaluate the Osstell ™mentor (Integration Diagnostics AB, Gothenburg, Sweden) as a diagnostic tool capable of discriminating between stable and mobile implants and to evaluate cut-off threshold ISQ value at implant placement that might be a predictive of osseointegration and up to 6 months post placement. Moreover, a correlation between ISQ values and the implants-mesial and distal bone density was carried out. Ten patients (8 men and 2 women) required extraction of maxillary anterior or premolar teeth and planned for immediate dental implants were accepted. A total of 12 Implant Direct's Screw Plant ™ implants (www.implantdirect.com) were placed immediately after extraction of teeth in the selected patients (one implant for each patient and only one patient received three implants). All implants were placed using a non-submerged technique. Immediately after placement of the implant, the Osstell ™ mentor was used for direct measurement of implant stability. Then RFA measurements were recorded at one, two, four and six months postoperative. Periapical digital radiographs were taken postoperatively at the predetermined time intervals. The ISQ values over time intervals, as well as, bone density in the mesial and distal sides of the implants were presented as means and standard deviation (SD) values. Paired t-test was used to study the changes by time. Pearson's correlation coefficient was used to determine significant correlations between ISQ values and the implants bone density %. The means ± SD of the ISQ at implant placement was 52.2 ± 5.2. The means of ISQ values at 1, 2, and 4 months after implants insertion were 58.3, 66.3, and 75.2 respectively, and at 6 months was 86.7. There was a statistically significant increase in the mean ISQ values through all periods ($P<0.001$). The lowest ISQ obtained at implant placement that might be predictive of osseointegration was 49. The results showed a positive correlation between ISQ values and mesial and distal bone density percentage. In conclusion, RFA with the Osstell monitor has been claimed to be useful for monitoring implant stability and osseointegration during the healing phase. The RFA method, as a diagnostic tool, was reliable in identifying implant stability and successful osseointegration for implants with an ISQ $\geq$ 49. The ISQ values increased gradually with time in correlation with the increased mesial and distal bone density percentage.

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***Key words**:* Clinical study, immediate dental implants, ISQ, implant primary stability, Resonance-frequency analysis

1. INTRODUCTION

Maintaining bone quality and quantity in the alveolar ridge during and after tooth extraction is critical for assuring good aesthetic and functional results and minimizing the need for grafting procedures prior to implant placement. Following tooth extraction, bone remodelling usually takes place with the final outcome of alveolar bone reduction in both height and width, Meredith(1998). The placement of immediate implants prevents bone resorption and preserves the alveolar crest at the extraction site. Single-rooted teeth, predominately incisors and premolars, have been the most frequent sites for immediate implants, Fonseca (2000); Ekfeldt et al., (1994).

Primary implant stability has been identified as a prerequisite to achieve osseointegration. The term *osseointegration* was defined as "the direct structural and functional connection between ordered living bone and the surface of a load-carrying implant." Osseointegration has also been defined in clinical terms as "a process in which clinically asymptomatic rigid fixation of alloplastic materials is achieved and maintained in bone during functional loading", Albrektsson et al.,(2000). Primary stability occurs at the time of

implant placement and is related to the level of primary bone contact, Cochran et al.,(1998). It is influenced by the length, geometry, and surface area of the implantand by the bone-to-implant contact area, Meredith (1998).Other factors include the ratio of cortical to trabecular bone and the placement technique. Secondary stability is the result of the formation of secondary bone contact of woven and then lamellar bone. During healing, as primary bone contact decreases, secondary bone contact increases, Cochran et al.,(1998). Primary and secondary stability in healed bone has typically been clinically assessed via tapping the implant in a lateral direction with 2 opposing mirror handles, Cochran et al.,(2002) .Although this is a widely practiced clinical technique, there is little evidence in the literature to suggest that this method is valid. A clearly perceived need for a quantitative method to measure implant stability exists, Meredith (1998).

Periotest or the Dental Fine Tester as objective measurements of primary stability has been proposed. However, their lack of resolution, poor sensitivity and susceptibility to operator variables has been criticized, Meredith (1998); Friberg et al.,(1993). Radiographs, in spite of their relatively good diagnostic accuracy in detecting bone level changes, are not sensitive enough to predict clinical implant instability with any certainty, Sunden et al.,(1995). Therefore, Resonance Frequency Analysis (RFA) has been introduced to provide an objective measurement of implant primary stability and to monitor implant stability over the healing period Meredith et al.,(1997a); Meredith et al.,(1997b); Friberg et al.,(1999b); Bischof et al.,(2004). Implant primary stability plays a key-role in achieving osseointegration. Distinct ranges of implant primary stability have been distinguished by the resonance frequency method, Jaffin, and Berman(1991); Meredith et al.,(1996); Meredith et al.,(1997c); Balleri et al.,(2002).Resonance frequency analysis is a method that involves the use of a small transducer attached to the implant abutment. This transducer acts as an electronic fork that vibrates and sends out a frequency to the jaw bone, Sullivan et al.,(1996); Young et al.,(2001); Dario et al.,(2002); Huang et al.,(2002); Huang et al.,(2003) . The resulting resonance frequency is translated into an index called Implant Stability Quotient (ISQ) and the appliance is known commercially as Osstell (www.osstell.com).

Therefore, it was hypothesized that determination of a primary stability threshold, provided in terms of a defined threshold ISQ value, might be relevant to predict the osseointegration prognosis of a given implant, Jaffin, and Berman(1991); Lazzaraet al.,(1996); Sennerby and Meredith(1998); Nkenke et al.,(2003) .

The feasibility of the RFA technique for implant stability measurement in human application has been proved in vivo animal models by Meredith et al.,(1996); Meredith et al.,(1997b); Meredith et al.,(1997c); Huang et al.,(2000); Bischof et al.,(2004), Glauser et al.,(2004) and in vitro studies by Huang et al.,(2003); Huang et al.,(2005). Nedir et al (2004) conducted a study to evaluate the Osstell as a diagnostic tool capable of differentiating between stable and mobile ITI implants, and to evaluate a cut-off threshold ISQ value obtained at implant placement (ISQ itv) that might be predictive of osseointegration. They concluded that implant stability could be reliably determined for implants with an ISQ itv $\geq$ 47 and all implants with ISQ itv $\geq$ 54 osseointegrated when immediately loaded. Lachmann et al.,(2006) conducted a study to evaluate reliability of the Osstell and Periotest devices in the assessment of implant stability and to perform a method comparison. Commercial dental implants were inserted into bovine rib segments of different anatomical origins and densities. The results showed that both RFA and Periotest were comparable and showed a strong association to each other and recommended for clinical use in the assessment of implant stability.

Therefore, the purpose of the current study was directed to evaluate the Osstell ™mentor (integration diagnostics AB, Gothenburg, Sweden. www.Osstell.com) as a diagnostic tool capable of discriminating between stable and mobile implants and to evaluate cut-off threshold ISQ value obtained at implant placement that might be a predictive of osseointegration. Also to evaluate the correlation between ISQ values and the implants mesial and distal bone density percentage (%).

2. MATERIALS AND METHODS

This study was designed to measure stability of immediate implants with RFA (Integration Diagnostics AB, Gothenburg, Sweden. www.Osstell.com) at the time of implant placement and up to the sixth postoperative month.

2.1. Materials:

2.1.1. Subjects:

Ten Patients (8 men and 2 women) were selected from the Outpatient Clinic, Department of Oral and Maxillofacial Surgery, Faculty of Oral and Dental Medicine, Cairo University.

Patients' ages ranged from 25 to 40 years. At the initial screening appointments, the subjects'

medical and dental histories were reviewed and inclusion/exclusion criteria (Figure 1) were confirmed, Barewal et al.,(2003).

1- Patient inclusion criteria

a. Age 18 years or older
b. Ability to understand and sign the informed consent prior to starting the study
c. Ability and willingness to comply with all study requirements
d. Adequate oral hygiene (defined as an average Modified Sulcus Bleeding Index of 1 or less and an average Modified Plaque index of 1 or less
e. Adequate bone volume to accommodate the planned endosseous dental implants (e.g., sufficient height such that the implant would not encroach on vital structures such as sinuses and sufficient width that the implant could be placed within the confines of the existing bone
f. If the patient was of childbearing potential, a negative pregnancy test within 1 week prior to surgery
g. Accepted teeth to be replaced with immediate implants are fractured teeth following trauma, teeth with vertical or horizontal root fracture and badly broken non restorable tooth. Those teeth should be free from periapical pathological lesions, free from any alveolar bone damage due to trauma and should have at least 3mm of sound bone present beyond the socket apices.

2. Patient exclusion criteria

a. Moderate or heavy smoking (more than 10 cigarettes per day) or tobacco chewing
b. History of alcoholism or drug abuse within the past 5 years
c. Severe bruxing or clenching habits
d. Untreated periodontitis
e. At risk for a surgical procedure
f. Presence of local inflammation
g. Uncontrolled diabetes
h. Current hematologic disorder or coumadin (or similar) therapy
i. History of leukocyte dysfunction and deficiencies
j. Metabolic bone disorders
k. History of renal failure
l. History of liver disease
m. Immunocompromised status, including HIV and herpes virus
n. Current steroid treatment, i.e., any person who within the last 2 years had received for 2 weeks a dose equivalent to 20 mg hydrocortisone
o. Current chemotherapy
p. History of radiation treatment to the head or neck
q. Physical limitations that would have interfered with patient's ability to exercise good oral hygiene on a regular basis
r. A need for grafting of bone or soft tissue at the time of implant placement
s. A need for submersion of implants for esthetic reasons

Figure 1: Inclusion and exclusion criteria for the present study.

Only patients requiring extraction of maxillary anterior or premolar teeth and planned for having immediate dental implants were accepted. Clinical and radiographic screening was used to limit the study to patients with sufficient bone quantity (Figure 2-4).

Figure 2: Preoperative periapical radiograph for a case showing fractured upper left central incisor

Figure 3: Photograph of a case showing badly broken non restorable upper left lateral incisor

Figure 4: Photograph of a case showing fractured upper right central incisor.

2.1.2. Implants:
A total of 12 Implant Direct's Screw Plant ™ implants (www.implantdirect.com) were used.

2.2. Methods:

2.2.1. Surgical Design:

12 Implant Direct's Screw Plant ™ implants were placed immediately after extraction of teeth in the selected patients (one implant for each patient and only one patient received three implants). Mesiodistal socket width and length were measured to help in selection of appropriate implant diameter and length with the aid of transparency guide template, provided by the implant manufactures. All implants were placed using a non-submerged technique, according to a strict surgical protocol following the

manufacturer's instructions.Immediately after the implant was placed, the Osstell ™ mentor for RFA measurements was used for direct measurement of implant stability. Then RFA measurements were recorded at one, two and four months after implants insertion and six months postoperative at time of loading of the implants .The implants were restored with ceramic crowns 6 months after insertion.

Two hours prior to surgery, 1gm Augmentin (875mg amoxicillin and 125 mg clavulanate potassium, GlaxoSmithKline S.A.E, El Salam city, Cairo, A.R.E.) was given orally to the patient as a prophylaxis against infection. The patient was anesthetized using a standard solution of 2% mepivacaine hydrochloride (The Alexandria Co. for Pharmaceuticals, Alexandria, Egypt) with 1: 20 000 levonordefrin. Infiltration or nerve block anaesthetic techniques were used. The oral cavity was rinsed with 0.2% chlorahexidine gluconate (Hexitol mouth wash, The Arab drug Co. Cairo, Egypt) for 30 seconds immediately before extraction to obtain aseptic environment.

A traumatic extraction was accomplished in order to protect and preserve the alveolar bone plates. In all cases, no bone or tooth fracture has been occurred during extraction (Figure 5).

Figure 5:
A photograph showing atraumatic upper right incisors and first premolar teeth extraction.

Following tooth extraction, the socket was debrided by small sized curette to remove any remnants of the periodontal tissue. Then copious irrigation of the socket with sterile solution was done. The socket was then dried and carefully inspected. The extracted root length as well as the socket depth and width was measured to estimate appropriately implant diameter and length. The implant diameter was selected according to the estimated mesiodistal and bucco-lingual dimensions of the empty socket. The implant length was selected according to the estimated root length of the extracted tooth. During selection of the implant length we took into consideration that, the implant length should be 3-4 mm longer than the root length of the extracted tooth to engage the apical 3-4 mm bone in the extraction socket whenever possible to obtain primary stability.

The extraction sockets were prepared to receive Implant Direct's Screw Plant ™ implants of 13mm length and 3.7, 4.7 and 5.7 mm diameter. All drilling procedures were completed using a high torque low-speed (800 rpm) surgical motor with an internal irrigation device using sterile saline solution. Internally irrigated stepped standard drills in different lengths and diameters were used until the desired implant diameter was reached. Then prepared sites were irrigated with normal saline to remove bone fragments. The implant was then threaded in place using the ratchet in a clockwise direction. The cover screw was screwed into the implant body to cover it. All implants were placed using a non-submerged technique. Implant Direct's Screw Plant ™ implants have 2mm extender retained by cover screw for use as a healing collar in that non-submerged technique. Therefore the healing collar was left exposed intra-orally to allow the commercially available transducer adapted to the Direct's Screw Plant ™ implants to hand-screwed into the implant as recommended by the manufacturer during RFA measurements. Augmentin 1gm orally every 12 hours to guard against infection and Oflam 50mg (Oflam lactab, Medical Union Pharm Co., Ismailia, Egypt) orally three times a day as analgesic were given to the patients for the following five days postoperative. Chlorahexidine 0.2% mouth rinse was used three times a day for the next 14 days postoperative. Removable prostheses were used for esthetic and social reasons but these prostheses were not pressing on the implant site and were out of occlusion.

Implant primary stability was first assessed clinically by finger pressure exerted on the implant-mount. If stable, the cut-off threshold ISQ value immediately after the implant placement; referring as the resonance frequency of the implant-bone complex, was measured with Osstell apparatus (Figures 6-8) using a commercially available transducer adapted to the Implant Direct's Screw Plant ™ implants.

Figure 6: A photograph showing Osstell ™ mentor measurement probe, power adapter and commercially available transducers.

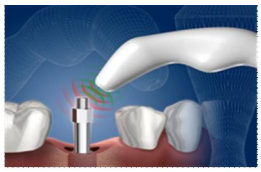

Figure 7: A diagrammatic photograph showing Osstell ™ mentor in use

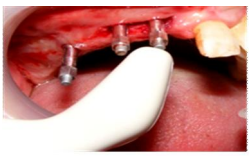

Figure 8: A photograph showing Osstell ™ mentor in use with the probe close held to the transducer during the pulsing time.

To perform the measurements, the cover screw was removed at each time interval and then the transducer was attached to the implant fixture and tightened directly onto the implant neck. Hold the probe of the Osstell ™ mentor device close to the transducer during the pulsing time (three short beeps). After that the probe can be taken away from the transducer. After the processing time the instrument beeps again (once), the small blue light turns on and the ISQ value was presented in the display . Repeat the measurement at a different rotational angle (45^0 – 90^0). To be able to measure both the lowest and the highest stability it is recommended to make a second measurement at a different rotational angle (45^0 – 90^0) degrees from the first . Readings were obtained 2 times to ensure repeatability of the instrument . Therefore, the cut-off threshold ISQ at implant placement was recorded that might be predictive of osseointegration. The cover screws were then replaced (Figures 9 and 10).

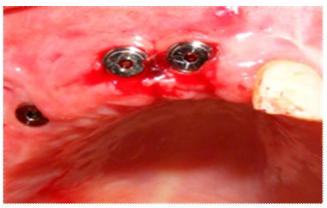

Figure 9: A photograph showing implants in their final position

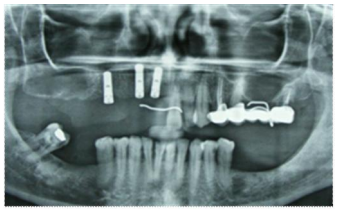

Figure 10: Immediate postoperative radiograph.

The ISQ value at each time point was further measured at 1, 2, and 4 months after implants insertion and 6 months; at time of loading of the implants. The RFA values, calculated from the peak amplitude, were represented in a quantitative unit called ISQ on a scale from 1 to 100. ISQ values were derived from the stiffness (N/µm) of the transducer/implant/ bone system and the calibration parameters of the transducer, Nedir et al.,(2004); Lachmann et al.,(2006). Classically, an increased ISQ value indicates increased stability, whereas decreased values indicate a decrease in implant stability according to previous studies by Nedir et al.,(2004); Lachmann et al.,(2006).

2.2.2. **Postoperative**

Postoperative periapical digital radiographs were taken by x-ray machine (Orix 70 dental intraoral x-ray unit, Italy) at the predetermined time intervals, using long cone paralleling technique to obtain reproducible radiographs at each follow-up interval. Customized bite acrylic template was fabricated for each patient and was used in conjunction with radiographic film holder system (Rinn's XCP film holder to hold the Photo-Stimuable Phosphor plate (PSP) or sensor in a fixed relation to the area to be examined). The sensor was held parallel to the implant long axis, and the

x-ray beam was directed perpendicular to the long axis of the implant. The exposure parameters were standardized for all patients (70 KVp, 8 mA, and 0.20 seconds). Bone density measurements were carried out using the specially designed Digora software (SPSS , Inc., Chicago, IL, USA) for Windows version 1.51, where 3 lines were drawn parallel and 1 mm apart from each other on both mesial and distal sides of the implant. The first line was drawn starting from the flute of the implant tangential to it till the base of the implant. The mean gray values (Pixels) of each line were collected and the means of the 3 line on each side were calculated for statistical analysis (Figure 11).

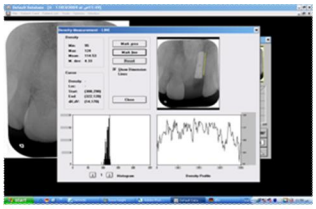

Figure 11: Digora® software for Windows used for measurements crestal bone density mesial and distal to implant in one case

Data of the implants ISQ values over time intervals as well as bone density values of the mesial and distal sides of the implants were presented as means and standard deviation (SD) values. Paired t-test was used to study the changes by time. Pearson's correlation coefficient was used to determine correlations between ISQ values and the implants mesial and distal bone density percentage (%). The significance level was set at $P \leq 0.05$. Statistical analysis was performed with SPSS 16.0 (Statistical Package for Scientific Studies; SPSS, Inc., Chicago, IL, USA) for Windows.

3. RESULTS

Postoperative healing was uneventful in all patients. There was no inflammation, no pain or suppuration around the implants. The means and standard deviation (SD) values for bone density % in the mesial and distal sides of the implants, as well as, ISQ values recorded immediately, 1,2,4, and 6 months postoperatively were shown in (Table 1) and (Figures12 and 13).

Table (1): Descriptive table showing the means and standard deviation (SD) values for bone density % in the mesial and distal sides as well as ISQ values

Period	Mesial bone density %		Distal bone density %		ISQ	
	Mean	SD	Mean	SD	Mean	SD
Immediate	87.8	0.5	87.9	0.6	52.2	5.2
1 month	88.5	0.5	88.6	0.4	58.3	7.3
2 months	88.9	0.5	89.1	0.5	66.3	5.8
4 months	90.7	0.6	91	0.6	75.2	3.7
6 months	94.1	0.5	94.5	0.6	86.7	1.7

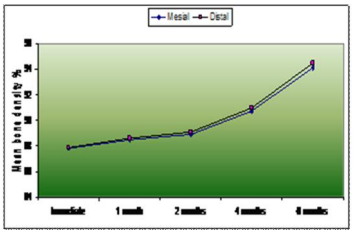

Figure 12: the means and standard deviation (SD) values for means bone density % in the mesial and distal sides of the implants

The means of the cut-off threshold ISQ at implant placement was 52.2 and SD was 5.2. The means of ISQ values at 1, 2, and 4 months after implants insertion were 58.3, 66.3, and 75.2 respectively, and at 6 months was 86.7, as shown in (Table 1).

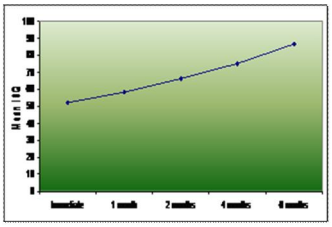

Figure 13: the means of ISQ values recorded immediately, 1, 2, 4 and 6 months postoperatively.

The mean differences, standard deviation (SD) values and results of paired t-test for the comparison between mean ISQ values at different periods were shown in (Table 2). There was a statistically significant increase in mean ISQ values through all periods (P<0.001). The mean differences in the ISQ values in (immediate –1month) was -6.2, (immediate – 2months) was -14.1, (immediate – 4months) was -23, and the mean differences between the final ISQ and that of implant placement (immediate – 6 months) was -34.5. The lowest ISQ obtained at implant placement that might be predictive of osseointegration was 49.

Table (2): The mean differences, standard deviation (SD) values and results of paired t-test for the comparison between mean ISQ values at different periods

Period	Mean difference	SD	*P*-value
Immediate – 1 month	-6.2	3.6	**<0.001***
Immediate – 2 months	-14.1	3.9	**<0.001***
Immediate – 4 months	-23	3.3	**<0.001***
Immediate – 6 months	-34.5	4.4	**<0.001***
1 month – 2 months	-7.9	2.7	**<0.001***
1 month – 4 months	-16.8	4.3	**<0.001***
1 month – 6 months	-28.3	6.2	**<0.001***
2 months – 4 months	-8.9	2.6	**<0.001***
2 months – 6 months	-20.4	4.8	**<0.001***
4 months – 6 months	-11.5	2.8	**<0.001***

**: Significant at P ≤ 0.05*

Mesial and distal bone density % in all implants at each time point was calculated as a percentage from the mean density of the native bone. The density in general increased gradually with time stating from 87.8% and 87.9% on the mesial and distal sides respectively immediately at implant insertion, till reaching 94.1% and 94.5% on the mesial and distal sides respectively after 6 months of implant insertion (Table 1 and Figure 5). The mean differences, standard deviation (SD) values and results of paired t-test for the comparison between mean mesial bone density % to the mean density of the native bone at different periods were shown in (Table 3).

Table (3): The mean differences, standard deviation (SD) values and results of paired t-test for the comparison between mean mesial bone density % at different periods

Period	Mean difference	SD	*P*-value
Immediate – 1month	-0.6	0.3	<0.001*
Immediate – 2 months	-1.1	0.3	<0.001*
Immediate – 4 months	-2.9	0.4	<0.001*
Immediate – 6 months	-6.3	0.5	<0.001*
1 month – 2 months	-0.5	0.1	<0.001*
1 month – 4 months	-2.3	0.3	<0.001*
1 month – 6 months	-5.7	0.4	<0.001*
2 months – 4 months	-1.8	0.2	<0.001*
2 months – 6 months	-5.2	0.4	<0.001*
4 months – 6 months	-3.4	0.4	<0.001*

**: Significant at P ≤ 0.05*

There was a statistically significant increase in mean mesial bone density % through all periods. Moreover, the mean differences, standard deviation (SD) values and results of paired t-test for the comparison between mean distal bone density % to the mean density of the native bone at different periods were shown in (Table 4). There was a statistically significant increase in mean mesial bone density % through all periods.

The correlation between ISQ values and mesial and distal bone density % was done. The results of Pearson's correlation coefficient for the correlation between ISQ values and mesial and distal bone density % were shown in (Table 5). There was a positive correlation between ISQ, mesial and distal bone density %. However, this correlation was not statistically significant through all periods.

Table (4): The mean differences, standard deviation (SD) values and results of paired t-test for the comparison between mean distal bone density % at different periods

Period	Mean difference	SD	*P*-value
Immediate – 1 month	-0.7	0.4	<0.001*
Immediate –2 months	-1.2	0.4	<0.001*
Immediate – 4 months	-3.1	0.5	<0.001*
Immediate –6 months	-6.6	0.5	<0.001*
1 month –2 months	-0.5	0.2	<0.001*
1 month – 4 months	-2.4	0.3	<0.001*
1 month – 6 months	-5.9	0.3	<0.001*
2 months – 4 months	-1.9	0.2	<0.001*
2 months – 6 months	-5.4	0.3	<0.001*
4 months – 6 months	-3.5	0.4	<0.001*

**: Significant at P ≤ 0.05*

Table (5): Results of Pearson's correlation coefficient for the correlation between ISQ values and mesial and distal bone density %

Period	Mesial bone density %		Distal bone density %	
	Correlation coefficient (r)	*P*-value	Correlation coefficient (r)	*P*-value
Immediate	0.275	**0.386**	0.250	**0.434**
1 month	0.007	**0.984**	0.052	**0.872**
2 months	0.263	**0.408**	0.239	**0.454**
4 months	0.107	**0.740**	0.081	**0.802**
6 months	0.242	**0.48**	0.145	**0.652**

4. DISCUSSION

The overall objective of this study was to quantify the early stability patterns of immediate implants. The Osstell device, which is essentially identical to the RFA developed by Meredith (1998) was able in the current study to measure the overall stiffness of the transducer/ implant/tissue system. The current study was in accordance with Friberg et al.,(1999a); and Friberg et al.,(1999b),who found that the Osstell device served as a sensitive tool for clinically monitoring implant stability during healing phase and up to 6 months postoperative. In RFA, the stiffness of the bone/implant interface was calculated from a resonance frequency as a reaction to oscillations exerted onto the implant/bone system. The implant was excited with an oscillating transducer screwed onto the implant and the resonance specific to the resonance system 'implant/ bone' captured electronically over a range of five to 15 kHz. The implant's own oscillation under a given transducer frequency was mainly dependent on the character of the implant's bony fixation. The unit of measurement in this approach was the ISQ that was calculated from the resonance frequency and ranged with increasing stiffness of the interface from 0 to 100 units, Sunden et al.,(1998); Meredith et al.,(1998).

Meredith et al.,(1997b); Bischof,et al.,(2004) found that determination of a primary stability threshold, provided in terms of a defined threshold ISQ value, might be relevant to predict the osseointegration prognosis of a given implant. Friberg et al.,(1999b) found that ISQ may vary between 40 and 80 and that the higher the ISQ, the higher the implant stability. A substantial increase or decrease in implant stability could be detected. In agreement with Friberg et al.,(1999b), the mean ISQ in the current study ranged from 52.2 (at time of implant insertion) to 86.7 (at the sixth postoperative month) and the lowest ISQ obtained at implant placement that might be predictive of osseointegration was 49. Moreover, there was a statistically significant increase in mean ISQ values through time intervals (P<0.001).

Olive and Aparicio(1990); Saadoun and LeGal (1992) reported that implants placed in softer bone failed more often than implants placed in denser bone. Moreover, Friberg et al.,(1999b) also reported that implants located in the posterior maxilla failed more often than implants placed in the anterior mandible. In contradiction to these reports, all implants in the current study that placed in the maxillary premolars area showed high success rate with increased ISQ values with increased implant- mesial and distal bone density percentage.

The implicit assumption is that implants undergoing osseointegration are supposed to increase their stability with time or at least maintain it. The effect of time in the current study on implant stability and osseointegration comes in agreement with Meredith et al.,(1997c); Meredith(1998); Friberg et al (1999 b); Friberg et al.,(1999a); they found that the stability of the implant was affected by healing time and the stiffness of the tissue adjacent to and surrounding the implant. Our findings showed that the time

factor on ISQ value was significant ($P \leq 0.05$). From baseline to 6 months postoperative, the stability patterns were noticeably different, especially at the fourth month (mean ISQ difference = -23; SD=3.3*; P <0.001)* and at the sixth month (mean ISQ difference = -34.5; SD= 4.4*; P <0.001)*.

Barewal, et al.,(2003) reported that the dynamic nature of bone during healing resulted in a change around the implant over time. Stability was required in this healing period and later during function to allow regeneration of bone to occur around the implant, rather than fibrous repair. In addition,Cochran et al.,(1998) found that primary stability occurred at the time of implant placement might be largely the result of the slightly larger diameter of the implant against the cut native bone surface, referred to as primary bone contact. Moreover, they found that secondary stability was the result of bony modelling. During this healing process, woven bone became lamellar bone, and secondary bone contact increased while primary bone contact decreased. In agreement with the findings of Cochran et al.,(1998) the current study examined the transition in levels of stability from the time of primary bone contact to the development of early secondary bone contact during the first two months of healing. The mean second month ISQ values (66.3) was higher than the mean baseline values (52.2). The mean ± SD mesial and distal bone density % at the second month postoperatively (88.9±0.5 for mesial side and 89.1±0.5) was higher than the mean baseline values.

No defined cut-off ISQ value has been validated until now through documented studies to determine the threshold value that discriminates between a mobile and a stable implant. Nedir et al.,(2004) designed a study to evaluate the Osstell as a diagnostic tool capable of differentiating between stable and mobile ITI implants and to evaluate a cut-off threshold implant ISQ value obtained at implant placement that might be predictive of osseointegration. They concluded that implants with an immediate ISQ $\geq$ 49 should reliably osseointegrated when they are left to heal for 3 months in the mandible and in the maxilla. These implants should require only minimal routine follow-up. On the other hand, less stable implants with an ISQ < 49 might still osseointegrated. Implants with an ISQ $\geq$ 70 seemed not to require scrutiny when implant stability decreases but then remained stable. Implants with an ISQ in the 60–65 range might remain stable or slightly decrease. Stability of the implants that have an ISQ > 60 should increase. A decrease of the ISQ value after 6 weeks of healing should warn the practitioner to put these implants under tighter scrutiny and decide on the relevance of unloading until regaining stability. In agreement with this findings ,the current study found that RFA as a diagnostic tool was reliable in identifying implant stability and predicting a successful osseointegration for implants with mean ISQ $\geq$ 52.2. In addition, we found that the highest ISQ obtained at implant placement that might be predictive of osseointegration was 54 and the lowest ISQ was 49. All implants in our study showed a statistically significant increase in mean ISQ values through all periods. Moreover, there was a statistical significant increase in mean mesial and distal bone density % to the mean density of the native bone through all periods. Failure of osseointegration was not recorded spite of the lowest recorded ISQ value was 49.

Szmukler-Moncler et al.,(2000) found that implants showing high primary stability with increased ISQ values over time and an earlier loading protocol may be indicated. However, it is difficult to advocate possible earlier loading protocols when stability levels and ISQ values were fluctuating in the first two months of healing. In agreement with these finding, only two implants in the current study showed a relative decrease in ISQ values at the fourth month and this decrease might be related to the minimal marginal bone loss that observed on radiographs. Possible earlier loading was advocated for these two implants until ISQ value increased at the sixth month postoperative.

In the present study, there was a positive correlation with time between mean ISQ values and mean mesial and distal bone density % indicating that the higher ISQ value with time was a result of the increased crestal bone density % to the mean density of the native bone. Our finding was in agreement with Cornelinil et al.,(2000) and Harmely et al., (2001) who found that increased bone density around dental implants gradually with time resulting in more osseointegration and increased ISQ values.

In summary, this study permitted an evaluation of the stability of the immediate implant using RFA during healing. The monthly visits allowed for regular and proper observation of the changes in bone density on periapical digital radiograph following implant placement and up to the sixth postoperative month.

In conclusion, RFA with the Osstell monitor has been claimed to be useful for monitoring implant osseointegration during the healing phase. The

RFA method, as a diagnostic tool, was reliable in identifying implant stability, predicting and monitoring osseointegration with time for immediate implants with an ISQ ≥ 49 recorded immediately after implant insertion. The ISQ values increased gradually with time in correlation with the increased bone density around implant.

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Photodegradation of o-cresol by ZnO under UV irradiation

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Abstract: The effective removal of o-cresol is currently both an environmental problem. This paper shows how degradation of o-cresol was carried out, in the presence of UV; by ZnO as photocatalyst. To measure the efficiency of photodegradation, the different variables studied included amount of photocatalyst, concentration of o-cresol and pH. The results showed photodegradation was favorable in the pH 6-9 range with amount of photocatalyst 2.5g/L, and o-cresol concentration 100ppm. The detected intermediates were 2-methylresorcinol, 2.5-hydroxybenzaldehyde and salicylaldehyde. TOC studies show that 94% of total organic carbon is removed from solution during irradiation time. This study indicates the great potential of ZnO to remove aqueous *o*-cresol under UV irradiation.
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Keywords: Photodegradation, *o*-cresol, Photoexcited, ZnO, Photocatalyst, UV-Irradiation

1. Introduction

Environmental organic pollution on a global scale have drawn scientists' attention to the vital need for environmentally clean and friendly chemical processes. Phenolic compounds such as o-cresol are widely used in manufacturing products such as cresol-based resin, herbicides, pharmaceuticals and surfactants (Shivaraman et al., 2000). Waste water from these industries contains a high concentration of o-cresol (Chang et al., 1995). *o*-cresol has been listed as the priority pollutant in the USA-EPA list (Callahan et al., 1979). Water solubility of o-cresol is above 24 at 25°C (Cooper, 1912). Therefore, o-cresol can be a significant threat to the environment (Kavitha et al., 2005; Pardeshi et al., 2008). Therefore the effective removal of o-cresol is currently an environmental and economic problem (Flox et al., 2007; Guyer, 1998). Photocatalysis is the field of current interest given its application in disinfecting drinking water and wastewater because they are plentiful, inexpensive, and environmentally friendly (Hoffmann et al., 1995; Marcì et al., 2003; Rajeshwar et al., 1995). Photocatalytic reaction is initiated when a photoexcited electron is promoted from the filled valence band of a semiconductor photocatalyst (SC) to the empty conduction band as the absorbed photon energy, *hv*, equals or exceeds the band gap of the photocatalyst. The electron-hole pair (e^--h^+) is generated at the surface of the photoexcited photocatalyst as shown below (Gaya et al., 2008)

Photoexcitation : $SC+h\nu \rightarrow e^- + h^+$ (1)
Adsorbed oxygen: $(O_2)ads + e^- \rightarrow O_2^{\bullet -}$ (2)
Ionization of water : $H_2O \rightarrow OH^- + H^+$ (3)
Protonation of superoxides : $O_2^{\bullet -} + H^+ \rightarrow HOO$ (4)
$HOO^{\bullet} + e^- \rightarrow HO_2^-$ (5)
$HOO^- + H^+ \rightarrow H_2O_2$ (6)
$H_2O_2 + e^- \rightarrow OH^- + OH^{\bullet}$ (7)
$H_2O + h^+ \rightarrow H^+ + OH^{\bullet}$ (8)

The hydroxyl ($^{\bullet}$OH) radical is a powerful and non selective oxidant for degrading organic compounds (Litter, 1999; Glaze, 1987; Peiró, 2001). ZnO is one of the most frequently used photocatalysts (Brezova et al., 1994; Maldotti et al., 2002) that removed several environmental contaminants under UV irradiation (Akyol et al., 2004; Daneshvar et al., 2007; Kansal et al., 2008; Sharma et al., 1995). Although, our previous work shows photodegradation of *m*-cresol by ZnO under visible-light irradiation (Abdollahi et al., 2011) However, no study has been conducted on aquatic *o*-cresol photodegradation using ZnO under UV irradiation. This work looks at the photodegradation of *o*-cresol by ZnO under UV irradiation. The effects of variables including *o*-cresol concentration, photocatalyst loading and pH on photodegradation efficiency were studied. In addition, the mineralisation and photoproducts were investigated by TOC measurement and Waters-Acquity ultra high pressure liquid chromatography (UPLC).

2. Material and Methods

ZnO (99%, Merck), *o*-cresol (99%, Merck), NaOH (99% Merck), H_2SO_4 (95%-97%) and other required chemicals were of reagent grade, obtained from Merck and were used without further purification. Various concentrations of *o*-cresol

solution were prepared using deionized water. Photodegradation of o-cresol was performed in binary batch photoreactors (Fig.1). To make the produced gas volatile (maybe CO_2), and increase solution fluidization and access oxygen for eq. (Haddad et al.), air was blown into the reaction solution using an air pump at a flow rate of $10m^3/h$. the blowing cooled air into the solution eliminated the lamp's heat effect and kept the temperature at around 25 °C. Magnetic stirring at 196 rpm was applied to make the suspension solution during the reaction. As showed in Fig.1, an UV-A lamp (6 watt) were used as light source for UV reactor. Throughout the experiment, the appropriate concentration of *o*-cresol solution was contacted with an appropriate amount of photocatalyst in the photoreactor. At specific time intervals, samples were withdrawn from the bulk solution, and filtered through 0.45µm PTFE filters. The concentration of *o*-cresol and residue organic carbon was measured using UV - visible spectrophotometer (shimadzu, uv-1650pc), TOC-VCSN analyzer, respectively. The used UPLC was fitted with an Acquity BEH phenyl C_{18} column (10 cm × 2.1 mm × 1.7 µm) and the detector wavelength was 271nm. The gradient elution method was applied over a 3-minute run time. The mobile phase was acetonitrile (65%)-water (35%), while a photodiode array (PDA) spectrometer operated at fixed detection wavelength for each experiment was used as a detector. The percentage degradation was calculated as follows:

$$\text{Photodegradation\%}=100[(C_0-C)/C_0] \quad (9)$$

Where C_0=initial concentration of *o*-cresol, C=concentration of *o*-cresol after photoirradiation. All photocatalytic degradation experiments were carried out in duplicate. Photodegradation of *o*-cresol was investigated in the absence of photocatalyst and at normal pH (7.37) as a background. Results show only 4.2% (2.4 mg/L) of *o*-cresol was photolysed in the absence of photocatalyst in present of UV irradiation. Therefore, *o*-cresol is relatively stable under visible light irradiation. The concentration of *o*-cresol was determined in the presence of photocatalyst in the dark. The removed amount of *o*-cresol was 7.51% (3.7mg/L) to 2.5 g/L of ZnO. The decreasing in *o*-cresol suggests it is adsorbed on the photocatalyst surface (Fox et al., 1993).

3. Results and discussion

Photocatalyst loading

A series of experiments were carried out by varying the amount of ZnO (0.5 to 3.0 g/l) to establish the effect of photocatalyst loading and to avoid unnecessary excess photocatalyst. The percent of photodegraded *o*-cresol (photodegradation%) vs. irradiation time was plotted in Fig. 2. The maximum photodegradation% was carried out in 2.5g/L of photocatalyst. This may be due to the fact that increasing the amount of photocatalyst increases the number of effective sites and consequently leads to enhanced production of $^{\bullet}OH$ radicals. Moreover, the number of *o*-cresol molecules adsorbed was increased owing to an increase in the number of photocatalyst particles, which causes increasing photodegradation (Fox et al., 1993). When the amount of photocatalyst was enhanced beyond optimum, however, photodegradation was not increased. The decrease in the photodegradation efficiency beyond the optimum photocatalyst loading can be attributed to increase of turbidity of the solution that reduces the light penetration through the solution known as light screening effect (Neppolian et al., 2002; Pardeshi et al., 2008). Furthermore, agglomeration and sedimentation of photocatalyst particles is also possible (Konstantinou et al., 2004). In such a condition, a part of the photocatalyst surface probably becomes unavailable for photon absorption and *o*-cresol adsorption, thus bringing little stimulation to the photocatalytic reaction.

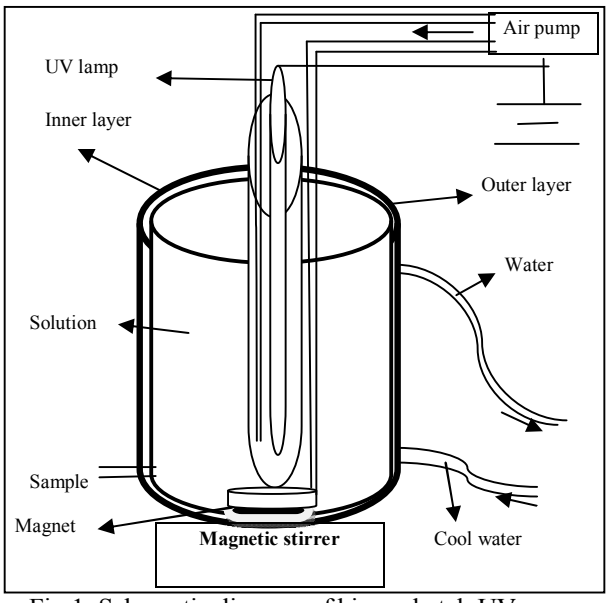


Fig.1. Schematic diagram of binary batch UV photoreactor

Effect of o-cresol concentration

The photocatalytic degradation of various *o*-cresol concentrations, 50-250ppm, was studied (Fig. 3). With an increase in concentration of *o*-cresol, photodegradation% was decreased. Table As observed the concentration of 100ppm completely and the sample of 150ppm more than 57% were

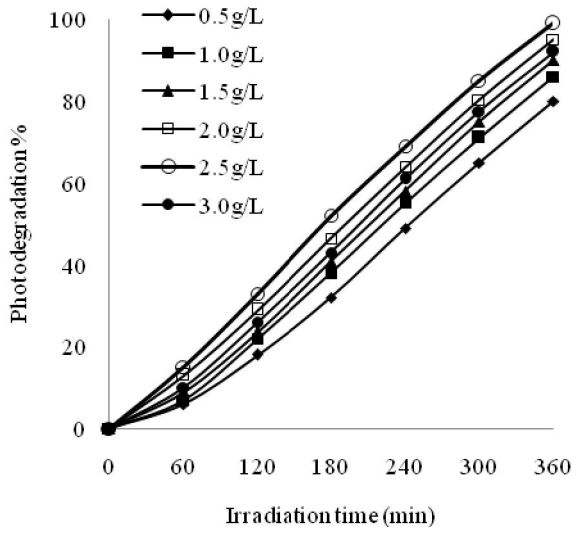


Fig.2 Effect of photocatalyst loading on photodegradation of o-cresol under UV irradiation, initial condition: o-cresols concentration = 100ppm, pH = 7.37

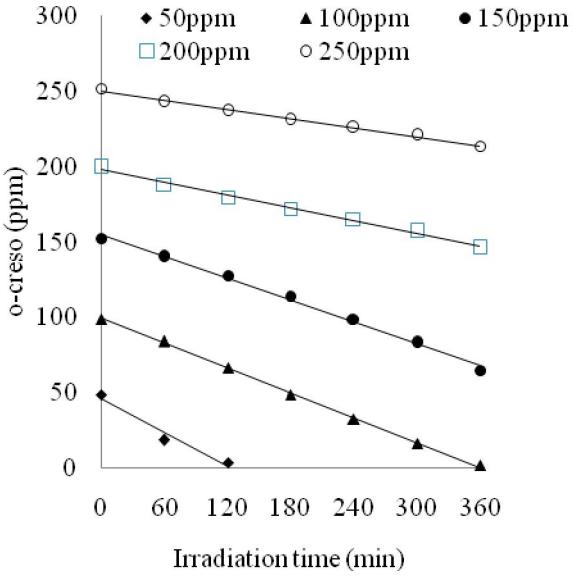


Fig. 3 Effect of o-cresol concentration on photodegradation under UV irradiation, initial condition: photocatalyst concentration = 2.5 g/L, and pH = 7.37

degraded however, with an increase in concentration of *o*-cresol, photodegradation% was decreased. At a high *o*-cresol concentration, the presumption is that active sites are covered by *o*-cresol and its intermediates that can cause reduced generation of e^--h^+, which reduces photodegradation efficiency (Konstantinou et al., 2004). The other possibility is that as the initial *o*-cresol concentration increases but the mass of photocatalyst, the intensity of light and illumination time are constant, then the •OH and O_2^{2-} species formed on the surface of photocatalyst are constant, so that the relative ratio of the •OH and O_2^{2-} for attacking *o*-cresol decreases and the photodegradation% decreases (Lathasree et al., 2004). Another factor which may be responsible for the reduction in photocatalytic degradation rate is the competition between adsorbed *o*-cresol and H_2O molecules for photodegraded h^+ (Gaya et al., 2008). Hence, the optimum *o*-cresol concentration for photodegradation is confirmed to be 100 ppm.

Effect of pH

The effect of pH on the photodegradation of *o*-cresol was studied (Fig.4). The optimum condition was used as in the previous experiment. As observed the amount of *o*-cresol photodegraded gradually increased with increasing pH from pH 6 to 9. Most organic compounds are removed on or near the surface of photocatalyst; therefore the activity of photocatalyst seriously depends on adsorption of amount of pollutant over photocatalysis (Abou-Helal et al., 2002; Anandan et al., 2007). Increasing photodegradation% may be due to the increase in adsorption of *o*-cresol on the photocatalyst surface. Increasing adsorption may be due to a decline of the electrostatic repulsive forces and increased interaction between photocatalyst surface (pH_{zpc}= 9) (Kosmulski, 2006) and *o*-cresol because with increasing pH the number of positive species (*o*-cresol-H^+) decreases. It has also been reported that in alkaline solution (pH 8-9), $^{\bullet}OH$ is easier to generate by oxidizing more OH^- available on the photocatalyst surface (Konstantinou et al., 2004). Thus, the photodegradation% is expected to increase with increasing pH due to an increase in available $^{\bullet}OH$ for the reaction. However, photodegradation% decreased at pH 10. This can be attributed to a reduction in the amount of *o*-cresol adsorbed on the catalyst surface at pH 10. It should also be noted that $^{\bullet}OH$ radicals are rapidly scavenged in high concentrations of hydroxyl ions and they have insufficient opportunity to react with the substrates (Davis et al., 1989). Hence, a drastic drop in the amount of *o*-cresol photodegraded by photocatalyst was observed at pH 10.

Mineralisation

Mineralisation is the main aim of photodegradation of *o*-cresol, followed by measuring the total organic carbon and total inorganic carbon (TIC). Fig. 5 shows the TOC and TIC values of *o*-cresol under UV irradiation. The amount of TOC was steadily decreased with increasing irradiation time, which indicates the decline of *o*-cresol intermediates when irradiation time is increased.

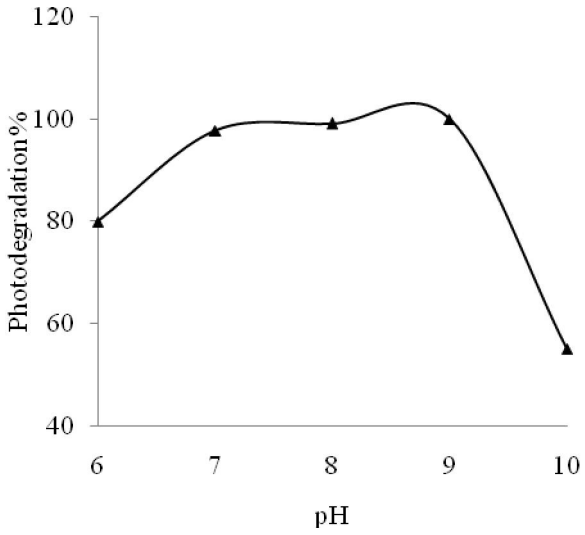


Fig.4. Photodegradation of o-cresol at variation initial pH under UV irradiation, initial condition, o-cresol concentrations = 100ppm, photocatalyst = 2.5g/L, during 6h.

This is most likely due to some organic carbon converting to inorganic carbon, for example, carbonate ions and after a few minutes TIC remained constant. According of the results, it can be concluded that 94% organic carbon is removed from o-cresol solution.

To confirm previous proposed pathway degradation of o-cresol in the presence of •OH radicals (Flox et al., 2007), determination of the some photoproducts of o-cresol photodegradation was carried out by UPLC. The chromatograms show many peaks. It may be due to the detection of carboxylic acids and other aromatic intermediates (Flox et al., 2007). Detected intermediates are 2-methylresorcinol, 2,5-hydroxybenzaldehyde and salicylaldehyde with retention time (R_t)=1.989, R_t=2.230 and 4.380 min at 60 to 120 min of irradiation time (Fig. 6).

Reusability

The reusability of photocatalyst was investigated in order to establish the stability of the *o*-cresol. While studying reuse of photocatalyst, all parameters including irradiation time, pH, *o*-cresol concentration, amount of photocatalyst and irradiation time were kept constant. The photocatalysis mixture was filtered, and washed five times with deionized water and then dried at 96°C in oven. Recovered photocatalyst was then reused five times as in the previous degradation process. Results show (Fig.7) no significant reduction in photocatalytic performance in photodegrading *o*-cresol, thus this indicates the stability of ZnO as a photocatalyst.

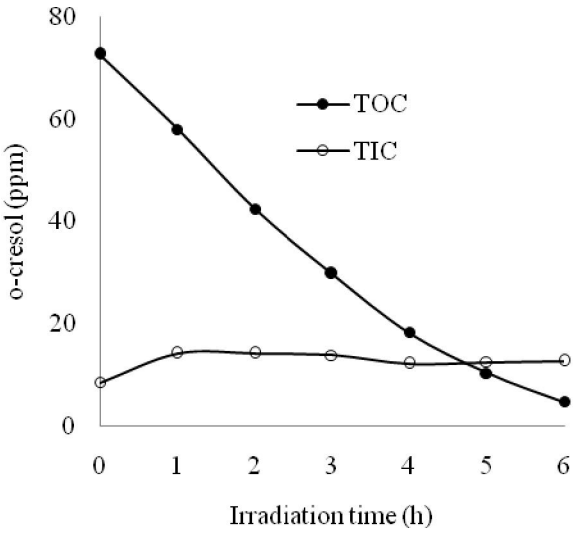


Fig.5 the amount of TOC and TIC during photodegradation of o-cresol under UV irradiation, initial condition; o-cresol concentration = 100 ppm, ZnO = 2.5g/L and pH = 7.37

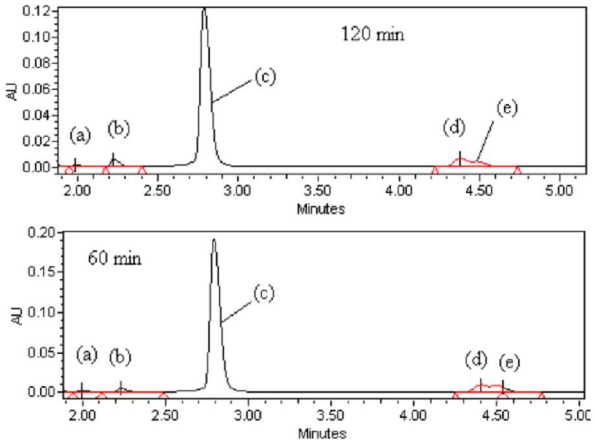
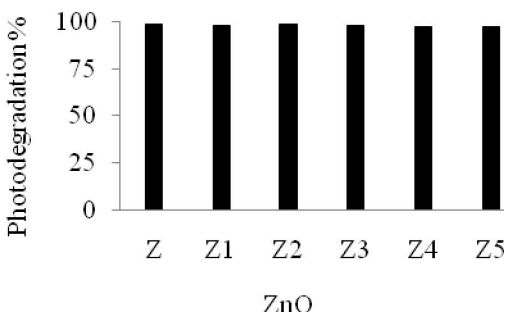


Fig. 6 selected UPLC chromatograms of o-cresol under UV irradiation that shows (a) 2-methylresorcinol, (b) 2,5-hydroxybenzaldehyde and (c) unknown (d) salicylaldehyde as intermediates and (e) o-cresol. Concentration of o-cresol= 50ppm at 60min



and 120min

Fig.7 Reusability of ZnO in photodegrading *o*-cresol solution under visible irradiation. Z is fresh ZnO cycle and Z1, Z2, Z3, Z4 and Z5 are reused ZnO cycle. Initial condition; Concentration of *o*-cresols = 100ppm, ZnO concentration = 2.5g/L, pH = 7.37, irradiation time = 6 h.

4. Conclusion

The photodegradation of *o*-cresol was carried out by ZnO under irradiation. The different variables studied included the amount of photocatalyst, concentration of *o*-cresol and pH impact on the efficiency of photodegradation. Under optimum conditions, the amount of photocatalyst and concentration of *o*-cresol were 2.5g/L and 100ppm, 25ppm. *O*-cresol photodegradation was favorable in the range pH 6-9. TOC studies shows that 94% of total organic carbon is removed from the solution during irradiation time. The detected intermediates are 2-methylresorcinol, 2.5-hydroxybenzaldehyde and salicylaldehyde. Reusability shows no significant reduction in photocatalytic performance in the photodegradation of *o*-cresol. This study indicates the great potential of ZnO to remove aqueous *o*-cresol under UV irradiation.

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The Cooperation Acid Rains Effect on to Historical Monuments, a Case Study from Canakkale Province; West Anatolia

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Abstract: Canakkale Monuments Observing data presented that the acid rains effect to in which has main $CaC0_3$ structure Apollo Smithion, Parion and Alexandria Troas according to particular by DTA graphics. The main reason of standing up of historical monuments in the region is caused by resistant greywacke structure, existence of quartz as a thermodynamically decisive form of SiO_2 has increased the resistance to time and acid rain in the region. Getting originated from industry and transportation which give rise to SO_x and NO_x inputs into the atmosphere on to Canakkale's historical monuments. Water loss coming out or high Uv energy which depends on temperature rise. Preserving measures maximum wet sediments in the seasons of autumn and winter rains effect coming from Balkans to study area, as a result to the density reach to the maximum level.
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Keywords: Acid rain, historical monument, environment, Canakkale, Turkey

1. Introduction

The effect of acid rains first became visible in Scandinavia in 1960s; contamination by acid was observed in thousands of lakes especially in Southern Scandinavia and in hundreds of lakes in Northern America. Also, it is known that water biota was affected and fish populations became reduced or extinct. Water contaminated by acid passed to soil and underground water, and this caused the rapid rusting of drinking water pipes in Scandinavia (Brundtland et al., 1987). On the other hand, in the middle of 1980s, the fresh waters in Sweden too acidified to support hydrophilic life and almost all of 1800 lakes became dead dormant (Kormondy 1996). Fish were determined to become extinct in the 24% of the lakes here (Environmental Resources Limited, 1983). The main reason for the interaction in these lakes is that the lakes had a catchment basin and is the possibility that snow waters could include 100 times more acid.

The effects of acid rains have increased in different environments in recent years. The studies regarding the emission values in the air at the surface of sea in the northeastern Atlantic proved to be over the previously- foreseen figures. This emission amount with the figures of 1995 is 1.37 million tons of sulfur dioxide and 1.94 million tons of nitrogen oxide (Acid News, 1997). Acid deposition continues to threaten many sensitive ecosystems, and analysts say deeper emissions cuts are needed to prevent future pollution from undoing the gains of the past 20 years (Malakoff D. 2010).

2. The Formation and Effects of Acid Rain

The pH of the atmosphere ranges between 7 >pH>5.6 because of the carbonic acid brought about by carbon dioxide. Rain water is of slightly acidic character in an environment not contaminated by any purity and its pH value is 5.6. With the fall of inflammable gases such as SOx, O_2, NOx, compound acid can come into being. The acid rains happen when the so-called gases rain onto the earth. pH can take place depending on biological and meteorological factors. The pH taking place in this way falls below 5.6. One of the main reasons for acid rains to affect wide range of areas is the Industrial Revolution. Another reason is the fact that the construction applications of elevated chimneys became wide-spread so as to rescue the atmosphere of the city from the emissions of sulfur dioxide. The factories and thermal power plants processing nickel and copper ores including sulfur compounds produced local problems in many countries; high chimneys were added to these plants as a solution. These chimneys the heights of which reach up to 250-300 meters (Boşgelmez, A. et al. 1997) reduced the local damage to a certain level, but produced negative effects to more larger areas.

Several industrial activities, fossil fuels used for heating in the houses, exhaust gases from the motor vehicles, and thermal power plants are polluting the air and are emitting sulfur dioxide, nitrogen oxide, particle matters and hydrocarbon. These pollutants that can hang on the air as dry and wet precipitates can sometimes be carried to far places and can compose sulfurous acid (H_2SO), sulfuric acid (H_2SO_4), nitric acid (HNO) by reacting

with water particles or other components in the atmosphere. The pollutants hanging in the air touch on the plant and soil surfaces with condensation. As the water contacts of dry compositions accumulating at the surface of geographical places increase, the negative effects increase (Harvey, 1989). The returns of them are dry and wet acid deposition. In wet deposition all the products in the atmosphere are carried in rain or snow in a solved way. In dry deposition, there is no rain and snow during the carrying of particles and gases in the atmosphere onto the earth ground. Mainly the effect of acid precipitation depends on: acid concentration, the period of exposure to concentration, the temperature of the environment and the moisture of rock, soil, body and air and cell structure.

The species in aquatic environment cannot live for a long time through its effect on aquatic environments. Many kinds of fish cannot reproduce when the pH reaches the value of 5.5. The old die and the young have difficulty in living. Fish die when the pH is 5.5 (Adriano and Johnson, 1989). It is not possible for fish to be alive more than a few minutes within the pH range of 3.0- 3.5, only some plants and inter vertebrate can exist (Alabaster and Llyod, 1979). For example, there is no fish in 20 thousand lakes of 100 thousand lakes in USA. Calcium hydroxide is still sprayed to get rid of the acidity in many lakes (Yılmaz, 1985). For instance, the more the pH value of water decreases, the more the species of Sphagnum can increase. 350 varieties of Sphagnum kind, which is of economic importance, grow in the marshy habitats and bring about wide meadows. Every year, new plants produce on the top, the lower part dies and may create thick layers of moss (Villee, 1972; Tanker et al, 1993).

Acid rains reflect on human metabolism as well. Heavy metals at high concentrations can be taken from drinking waters carried by pipe lines made of lead or copper. For example, the intake of copper at a high concentration is reported to give rise to diarrhea in small children. In the chain of aquatic food, the intake of heavy metals such as mercury and cadmium can be under discussion (Environmental Resources Limited, 1983). The acute and chronic effects of sulfur dioxide are in question. In acute effects, there are some reactions that cause the widening of capillaries and leakage of liquid. The general physiological reaction that happens with sulfur dioxide resembles to allergic asthma. In chronic effects, sulfur dioxide becomes included in lung diseases such as chronic bronchitis and pulmonary fibrosis and makes the symptoms aggravated by causing a decline in the pulmonary function through irritation and by increasing the burden on the heart (Kupchella and Hyland, 1993).

Acid rains are directly harmful on trees and other living beings in the forest ecosystem as well as their causing them to be affected negatively with the harmful effects which they produce in the roots by deteriorating the natural features of the soil. For this reason, the trees the nourishing relations of which are deteriorated die in the event that the negative effects continue or become intensified (Charles, 1995). In a survey made at Kaz Dağları district in the Marmara Region, acid rains were determined to cause leaf burns especially in a kind of pine (*Pinus nigra*) (Bayçu, 1997). Leaves are plants digesting and the most important respiration organs. Plants produce sugar and amino acids shortly organic materials by combining water and CO_2 in their bodies with sun shine. Plants can live feed, grow and produce fruit if the carbohydrates produced as the result of CO_2 digestion is more than those consumed through respiration (Carlson and Haines, 1989; Chew et al., 1980). Thus this effect on leaves causes product deficiency, leading to financial losses. However, the resistance of plants is also important. For example, it is observed that olive plants in the field soils show higher tolerance to the damage, while those growing on the slopes or mountainous places show lower tolerance. Trees in fertile soils with deep profile which can be watered and fertilized are more resistant to acid (Onoğur and Çaylak, 1989).

Its effect on soils and graywackes/rocks (kayaçlar) is quite important. According to Kantarcı (1995) and Kasap (1995), the acid accumulation in the soil affects some abnormalities in the system of soil and plant because it impairs the chemical structure of the soil. The concentrations of earth-related elements in the dry summer season lasting long and marine salt elements due to their near lines were found high. In the study, even though SO_4^{2-}, NO_3^- are high, H+ ion proved to be low because of neutralization. Marked differences were observed in the short (daily) and long (seasonal) period in the element and ion concentrations. Salt-related element such as Na and C1 increased due to stronger wind in winter. In the summer period, on the other hand, the soil's being dry and soil cultivation in the area together with the salt from Africa give rise to coming out of the concentrations of the soil-related ions such as $A1^{3+}$ and Fe^{3+} (Al-Momani et. al., 1998). For example, in a study carried out in Ankara, cation (H^+, Ca^{2+}, $NH4^+$) and anion (SO_4^{2-}, NO_3^-, Cl^-) analyses of the samples taken in the lab were carried out and as a result of the analysis, it was determined that pH value was under 5.6 in 23% of the rainfalls. Ion changes of sulphate, nitrate and calcium are excessive in Ankara rainfalls (Tuncel and Ungör, 1996). The acid deposited in the soil may become harmful when they cannot become cushioned by basic ions. In fact, basic

compounds subscribe to soil in the consequence of the abrasion of the rocks around; that's why, pH value of the soil reduces when unit rate of acid exceeds the rate of abrasion. Aluminum ions become in free position when the soil's pH value falls below 4.2 and they are absorbed by the plants like nutrients and very serious damages arise. When pH value falls below 3, iron ions damage the trees by becoming free. Very old main greywacke/rocks negatively affect meadow and forest plantation, and also aquatic eco-systems by causing their acidification because these rocks contain nutrients set free due to acid (Kinniburgh and Edmunds, 1984; Hornung et. al., 1995). At the same time, it is observed that in the studies carried out, geographical rock structure and the characteristics belonging to that area affect the acidic density and thus the natural water sources are affected (Stevens et al., 1997).

3. The research field and historical objects subject to research

Çanakkale is one of the two cities which is in the northwest of Turkey and located between 25'' 35' and 27'' 45' meridians and 38'' 30' and 40'' 45' parallels, and is situated in Asia and Europe with its lands on Gallipoli Peninsula and Biga Peninsula and which has lands on two continents. This city has a location at the geographical place where the Turks carried out the first conquest. The city is surrounded by Balıkesir in the East and Southeast, the Aegean Sea in the West, the city of Edirne in the Northwest, and Tekirdağ and the Marmara Sea in the North, and has a geographical space of 9.737 km^2. The study was conducted in the city that constitutes 1.29% of Turkey's acreage, having coast of 671 km to the sea bearing the transition climate between the Mediterranean climate and the Black Sea climate. It is the city that has 11 counties, 34 municipalities and 568 villages and whose total population is 464.957 and having the annual population growth of 7.3%. The city's population density is 47 and it hosted many civilizations in the course of history.

There are a lot of historical objects belonging to Hellenistic, Byzantium, Seljuk, the Ottomans and modern Turkey in the city. Some objects determined to be protected as historical heritage in the city of Çanakkale are as follows.

4. Material and Method

A study was carried out in the form of the land survey that is in the supportive quality for "The Studies of the Protection of Natural, Historical and Cultural Values" formed within the scope of the Studies of the Preparation of National Environment Action Plan, literature review and in-place observation. The method of problem solving which has emphasis of the anticipation of difficulty, the definition of the problem, the determination of the observable examiners and their evaluation were used. Collection of the samples from the historical objects found throughout the city between the dates of 05.01.2003 and 19.01.2003 and the study of the land were carried out. The analytical supply presented in this report are intended to aid researchers in the assessment of the effects of acidic deposition on building materials after collected-samples phases:

a) The determination of thermal analysis, and
b) The infrared spectroscopy were made in MTA-Ankara laboratories,
c) Determination of moisture,
d) Solubilization of the samples,
e) The loss of weight, and
f) Determinations of pH effect were made in ÇOMU Chemistry Department Laboratory.
g) Determination of the effect of temperature was carried out in Kale Ceramic/Semedeli

1- Chemical and crystal components
% SiO_2, CaO, Al_2O_3, Fe_2O_3, MgO, ZnO, K_2O
2- Mineralogical components
(boytovnit, calcite, quartz, albite, dolomite, tremolite, muscovite, etc.)
3- The examination of the samples' ionization values in pH2 - pH9 variability (at 25 ^oC).
4- in 30 -120 minute at 25 ^oC (weight loss)
5- in ph5 5 ^oC, 10 ^oC, 15 ^oC, 20 ^oC , 25 ^oC for 1 hour (weight loss)

The samples were not symbolized. The samples grinded in the scattered form in a homogeneous way in the values under the 205 MESH measurement unit were compressed and were contaminated with Shimatzu XRF 1700 device in XRF fluorescence Spectrometer. In addition, thermal analysis acidic interaction was made.

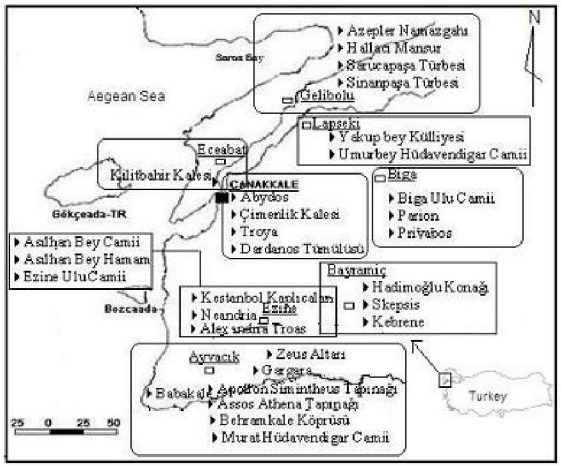

Figure 1. The Historical Sample Stations in Çanakkale Province

5. Schedule and the other components

Operation Program:

a) Compilation of source and literature
b) Determination of the historical objects to be protected in Çanakkale
c) Examination of the land
d) Managerial and institutional structures
e) Regulations
f) Financial and economic frames
g) The problems resulting from societal, cultural and political structure
h) The attempts to generalize the protection consciousness and protection education

6. Findings and Interpretation

In the construction of the historical objects in the city of Çanakkale, the materials in the area were used in general. The abundance of mine reserves that are the return of the present geological structure is related to its magmatic and the tectonic characteristics. As the result of the studies carried out in the land, it is emphasized that there may be a ore potential of 15-20 tons in terms of copper, lead-zinc. However, this potential was accumulated in a restricted area and it has spread to a vast area. In becoming ore, Paleozoic old magmatic are dense in acidic copper, lead, zinc, tungsten, molybdenum and iron and Tertiary old magmatic are dense in lead, zinc, antimony and quicksilver. In radio metric carbon dating carried out by C-14 method, it was determined that the metallic mine reserves have been operated since 3000 years. For example, it is estimated that the golden ore found in the Oligocene old silisilica tufas near the historical city of Truva was also being exploited 2455±70 ago. It was discovered that production was being made in the lead ore near Yenice Bekten village 2220±45 years ago (Ilgar R., 2004). It was mentioned that the constructors who live around Çanakkale attended to the building of 'Al-Aqsa Mosque' (Kural, 1988.) In brief the history of the region is rich in monument supplies and building and this also turned into application. A wealth of historical monuments is affected and unspoiled.

The effects of acid rains on the supplies used in building historical monuments are as follows.

Sulphat sulfur which can be in the state of Calcium, iron, copper and magnesium salts is in the state of loose crystal. The amount of sulphat coal in coal is quite little, but it can increase by surface deterioration when it contacts with air. Sulphat sulfur may not cause a problem in the purification of coal because it dissolves in water (Doğan et all, 1991). The kind of the Stone used is more the marble which is the most decisive form of mineral of CaC03 thermodynamically. Marble is a metamorphic greywacke. While marble is the big crystalline form of calcite, limestone is the small crystalline form of calcite and it has got a more porous structure. Because this difference affects the appearance and usage and also the marble is shinier, it has been used for minor historical buildings. In compliance with the results of the mineralogical studies made on the historical monuments taking place in the region, more limestone has been used as seen in the findings.

Figure 2. A Sample Exposed to Deterioration By Acid From The Research Field: The Column in Koçali Quarry

It is unavoidable that acid interaction is seen in the form of dry sediments except the direct effect of acid rains inside the available structure, because H_2O may join to the atmosphere and oxidation may take place. The generating possibility of this situation is strengthened by the following metrological data.

That the rains in the city take place in winter, as seen in the following table increases $S0_2$ and NO_2 relevant rise to acid rain. The findings got relevant to interaction of historical monuments which has the structure of the abovementioned greywacke with the acid and their mass losses are at appendices.

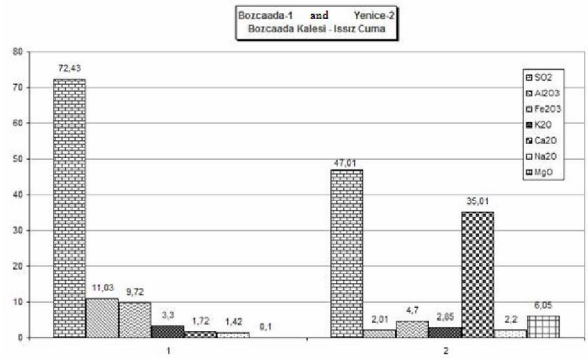

Figure 3. Mineralogical Analysis in The Historical Monuments of Bozcaada and Yenice Region

Great number of exothermic reactions have been seen in thermal analysis tests made on the samples taken from historical monuments. The

existence of the peaks in the reaction stands out. Mass loss and energy defect's occurrence has been noticed. There is water loss coming out or high Uv energy which depends on temperature rise. This condition is observed from DTA graphics. Consequently, It has been determined that acid rains have given rise to change relevant to degradation on the historical monuments in that region. This situation stands out more in Apollo Smithion, Parion and Alexandria Troas in particular.

The main reason of standing up of historical monuments in the region is caused by resistant greywacke structure.

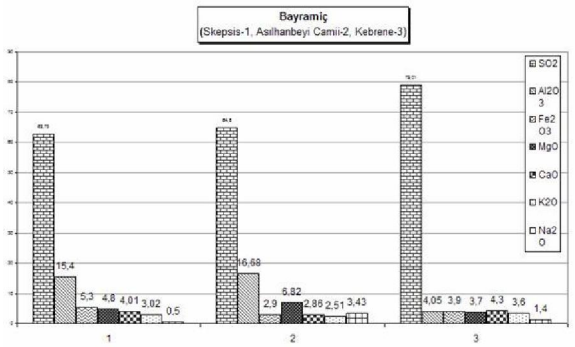

Figure 4. Mineralogical Analysis in the Historical Monuments of Bayramiç Region

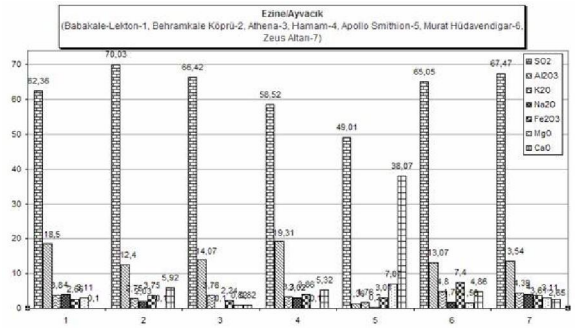

Figure 5. The Mineralogical Analysis in The Historical Monuments of Ezine and Ayvacık

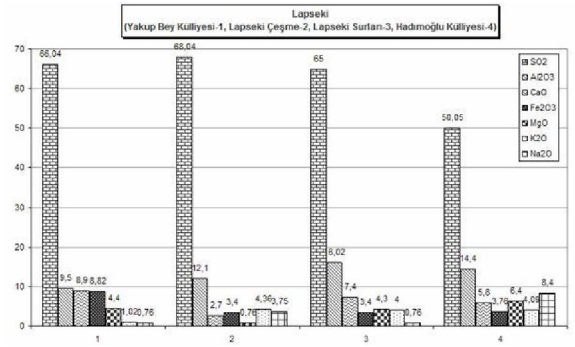

Figure 6. The Mineralogical Analysis in the Historical Monuments of Lapseki Region

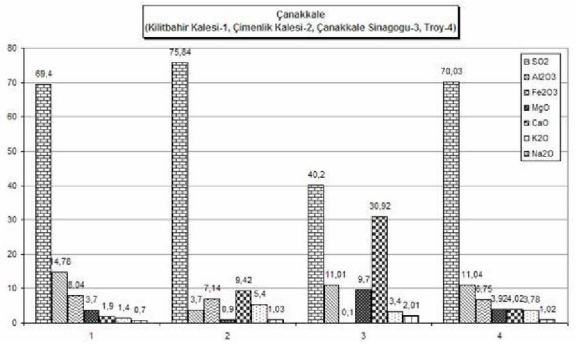

Figure 7. The Mineralogical Analysis in The Historical Monuments of Çanakkale and area's

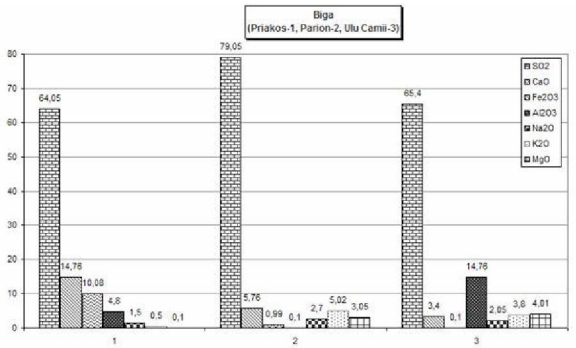

Figure 8. The Mineralogical Analysis in the Historical Monuments of Biga Region

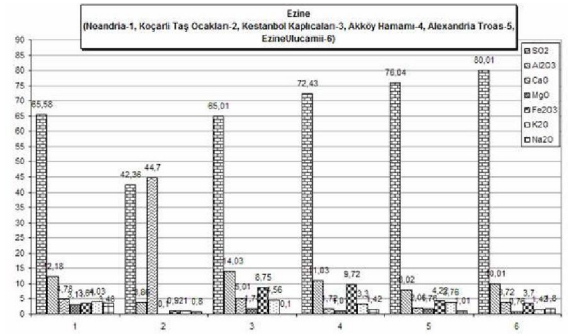

Figure 9. The Mineralogical Analysis in the Historical Monuments of Ezine

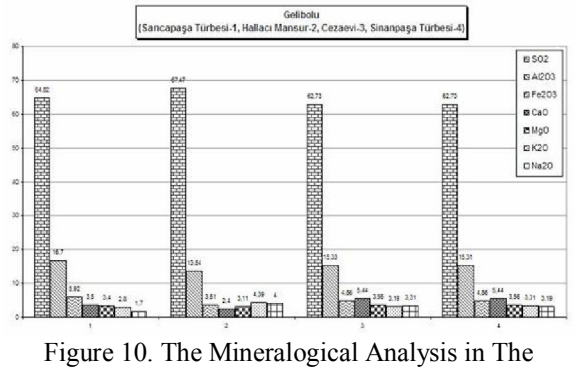

Figure 10. The Mineralogical Analysis in The Historical Monuments of Gelibolu

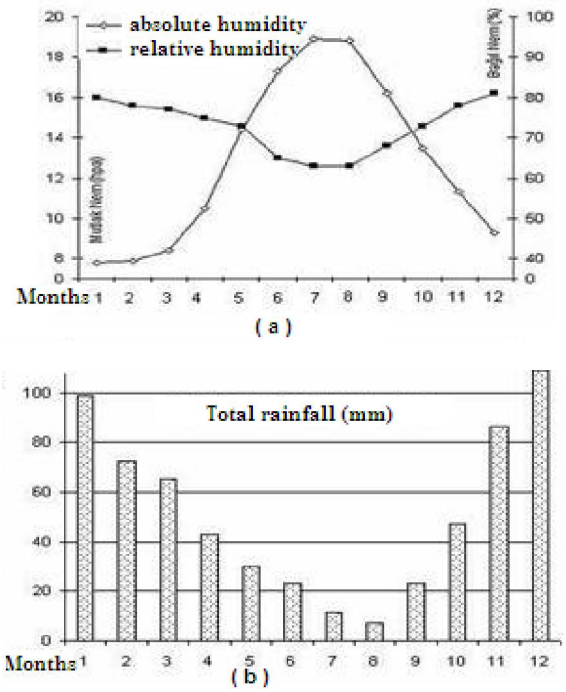

Figure (a-b) 11. The Moisture and Rain Condition Affecting Acid Rains in The Province (DMI, 1970-2002)

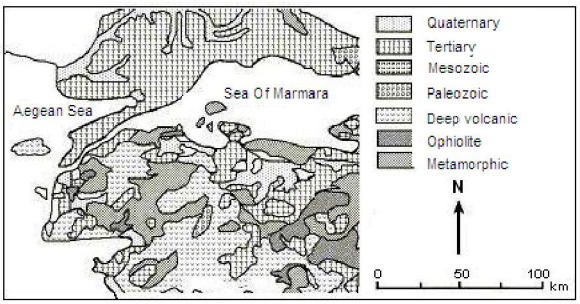

Figure 12. The Geological Structure of the Province of Çanakkale

There is resistance of the historical monuments to the acidification due to volcanic and metamorphic residing commonly in the structure of Biga peninsula. Sandstone takes place as a sedimentary greywacke. The dominance of greywacke with $CaC0_3$ is quite important. But sand particles include quartz herewith. Beside this, the existence of quartz as a thermodynamically decisive form of SiO_2 has increased the resistance to time and acid.

7. Recommendations

The density of greywacke with calcium carbonat of the historical monuments in the province of Çanakkale is great. The situation rises in the historical places of Çanakkale city center and nearby. This neutralizes with acid in the historical monuments. While the effect of acid is being absorbed mass and weight loss generates. It will be useful for dry savings to be prevented to hinder this situation. Inputs originated from industry and transportation which give rise to SO_X and NO_X inputs into the atmosphere where the historical pieces are must be prevented. Preserving measures must be taken for wet sediments. As a precaution, roofing and topping can be preferred in the seasons of autumn and winter the density reach to the maximum level. To that end, it is suggested for many international councils and nature preserving organizations to secure national historical monuments and for at least 10% or 12% of the total area taking up space in the ecosystem to be secured (McNeely J. A. and Miller K.R.1983, Noss R. F, 1996). If the success is achieved the territory which is called as the national park or the similar territory secured can be tripled (World Resources, 1996). It will be useful for this condition to be taken into consideration in the example of Çanakkale.

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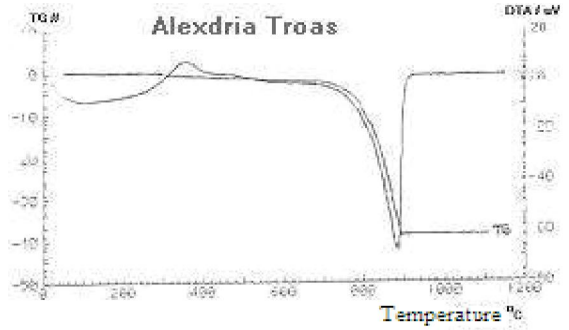
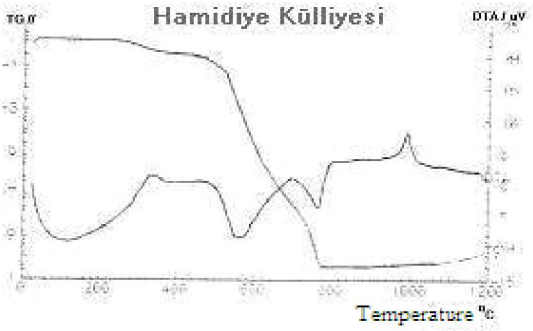
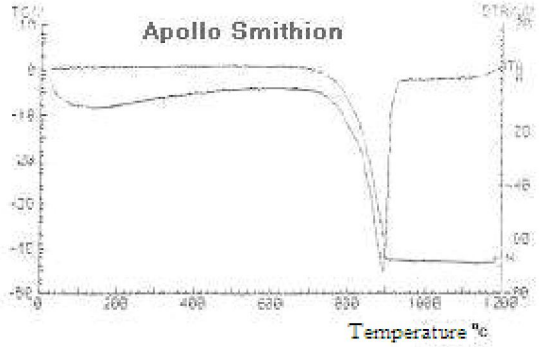
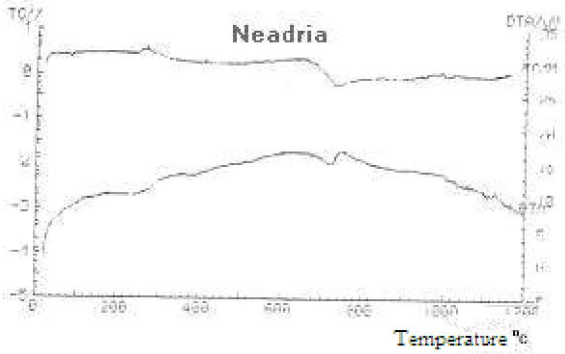
9. Appendix

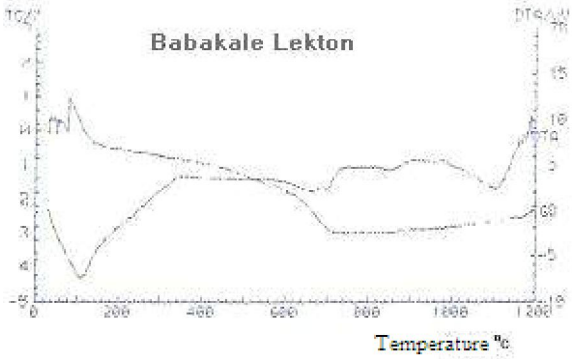
App.1. Rain Interaction Levels Belonging to Historical Monuments

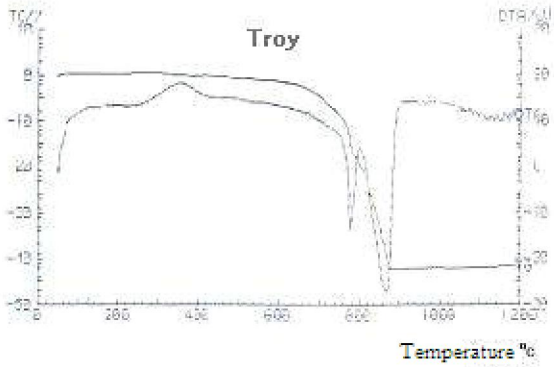


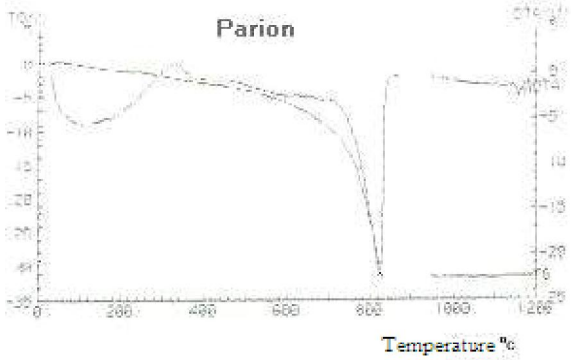


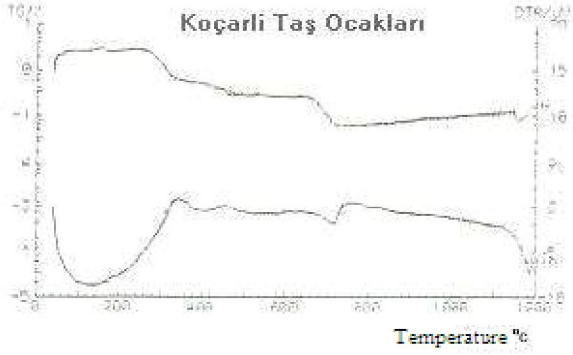












Sustainable Agriculture in Malaysia: Implication for Extension Workers

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Abstract: A global survey revealed that at present sustainable agriculture is implemented on only 3 percent of the total farming land in Asia, Africa, and Latin America. Researchers reported some obstacle to adoption of sustainable agricultural practices such as information on sustainable practices, economic factors, education and information, resistance to change, barriers related to sustainable agriculture technologies, social context, financial and material infrastructure and land tenure constraints. One of the main barriers to adoption of sustainable agriculture reported by farm producers and extension workers is lack of available information. Hence agricultural extension workers as information provider play a vital role for achievement of sustainable agriculture. The challenge extension workers faces are that of promoting sustainable agriculture to encourage farm producers adopt the program. The success of sustainable agricultural program depends on training and education of farm producers. The purpose of this paper is to explore whether extension workers have been sufficiently oriented themselves to their responsibility on sustainable agriculture or not.

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Keywords: Agricultural extension. sustainable agriculture. extension workers.

1.Introduction

Conventional agricultural practices have resulted in land, water, and air pollution, as well as loss of soil (Roberts, 1995). Environmental degradation, exclusion of small family farms from agriculture, poverty, lack of access to farm inputs and information and policy failures are among the main items that endanger agricultural sustainably. Efforts to raise productivity through pesticides and chemical fertilizers have been caused serious environmental issues (Wilson, 2000). These conditions promote sustainable agriculture. Sustainable agriculture continues to be emphasized to ensure that the well being of the present generation is not met at the expense of future generation (Shamsuddin and Wang, 2007). The primary assumption in sustainable agriculture is to develop farming system that promotes farming profits, agro-ecosystem and local communities. In contrast, unsustainable practices focus only on farm profit. Sustainable agriculture is defined as fruitful management of the agricultural resources to fulfill human needs, to preserve the environment, and enhance biological resources (Chikwendu and Arokoyo 1997). William (2000) defined economically sound, environmentally protective, and socially acceptable as three components of sustainable agriculture. Sustainable agriculture must be environmentally, economically and socially balanced farming systems that conserve the resources for the next generations (Bell, 2001). Hence the three major goals of sustainable agriculture are economic efficiency, social responsibility and environmental quality, (Fairweather & Campbell 2003). The transformation of conventional agricultural practices into sustainable agricultural system requires that farm producers adopt sustainable agricultural practices. Since sustainable agricultural system is an information intensive system hence agricultural extension plays a key role in assisting farm producers to adopt the sustainable agricultural practices.

Sustainable agriculture in Malaysia

Sustainable agriculture in developing countries such as Malaysia stress food security and sustainability of smallholders' livelihoods. Malaysia is an agricultural country that it is fast developing into an industrial country. This country has 4.06 million hectares of farm lands which 80% of this land planted with industrial crops (Murad et al, 2008). Malaysian government's policy towards agriculture emphasizes on increasing production, in order to achieve food self-sufficiency and to expand exports. Approximately 90% of farm producers in the food sector are smallholders with uneconomic-sized farms, the cost of production of these smallholders is high, with low input, low yield and poor quality of products. Agriculture in Malaysia has been relied on conventional methods of farming. Due to the rapid expansion in crop production there has been a corresponding increase in fertilizer use by farm producers. The government has been helping farm producers with fertilizer subsidies in order to improve their income and alleviate poverty (FAO, 2004). It is expected that the usage of mineral fertilizers will continue to rise if the intensity of production continue

to increase. Mineral fertilizers account for more than 90 percent of fertilizers used by all types of farming systems in Malaysia. However, in recent years positive steps undertake by the government agencies to reduce chemical fertilizers and optimize the use of resource on a sustainable base moving towards sustainable agriculture. Towards this objective, attention has been given on sustainable agricultural productions. Practices such as Integrated farming systems (IFS), Good Agricultural Practices (GAP) and Organic Farming (OF) are being promoted vigorously by the Department of Agriculture (DOA) in order to reduce dependence on mineral fertilizers for crop production. Currently there is a cooperative effort to concern the use of fertilizers and to place more emphasis on the usage of organic fertilizers (Ahmad, 2001). Under the Ninth Malaysia Plan (2006-2010) the government was targeting organic farming industry to be worth MYR 800 million in 5 years time. The Ministry of Agriculture planed to have 20,000 hectares under organic farming methods by 2010 (Murad et al, 2008). At present, these practices are not accepted and properly practiced by majority of farm producers in the Malaysian smallholders sector. For that purpose the mission of the Department of Agriculture (DOA) currently includes provision of extension services to the farm producers in relation to sustainable agricultural practices. In general the effectiveness of extension education is dependent on the ability of extension workers who must be competent and qualified as the whole extension process is dependent on them to transfer sustainable practices to farm producers. However despite frequent attention in recent years on sustainable agricultural practices, the adoption of sustainable practices such as GAP has been slow by farm producers (Othman, 2006). Murad et al, (2008) reported that although the Malaysian scheme on Good Agricultural Practices was launched on 31 January 2002 and the Department of Agriculture has already taken positive steps to promote some of the program, however majority of the farm producers were reluctant to practice the program. Murad et al, (2008) further contended that Malaysian agricultural policies are supportive for sustainable agricultural practice however the present agricultural practices in Malaysia differ from the standard of sustainability. Hock (1999) reported that more components of sustainable agriculture are expected to be included by government agencies in Malaysian agricultural sectors.

Extension Workers' Responsiveness of Sustainable Agriculture

The transformation of conventional agricultural practices into sustainable agricultural system requires that farm producers to adopt sustainable agricultural practices. Hence, extension workers could play a main role in assisting farm producers in their decision making process regarding the adoption of sustainable agricultural practices. Although sustainable agriculture was recognized very important, extension workers' knowledge and support for the concept is not favorable (Minarvic and Mureller, 2000). According to Al-Subaiee et al. (2005) the first step in sustainable agriculture plans is training extension workers to develop their understanding, qualifications and ability to teach farm producers. Nonetheless researchers show that extension workers have problems in the first step of understanding the concept of sustainability (Chizari et al., 2006; Allahyari et al., 2008). Agunga (1995) described that extension workers in Ohio did not have a firm understanding of sustainable agriculture. Hence they were less interested in promoting sustainable agriculture. Conner and Kolodinsky (1997) revealed that extension workers in New England also have doubting attitude toward sustainable agriculture. This finding implies that sometimes, extension workers' skepticism toward sustainable agriculture may be due to their inadequate knowledge about sustainable agricultural practices. Finding of study indicated that attitude of Iranian agricultural extension professionals including extension workers is not in favorable situation (Allahyari et al., 2008). Results of study conducted by Minarvic and Mureller (2000) indicated extension workers' attitudes reflected that they realized the importance of the sustainable agricultural concept and were knowledgeable about it, but when asked about actions taken to apply a systems thinking philosophy as one concept to define their attitudes towards sustainable agricultuer, there was no evidence of strong extension efforts. The challenge extension faces is that of promoting sustainable development. Adoption of sustainable practices by farm producers is the key to transform agriculture into a sustainable system. In a global survey, Pretty and Hine (2001) mentioned that at present sustainable agriculture is implemented on only 3 percent of the total farming land in Asia, Africa, and Latin America. One of the main barriers to adoption of sustainable agriculture is the lack of accessible information for farm producers and how to disseminate this information to them (Singh and Osawaru, 1990). Similarly, Barrow, Chan and Masron (2010) reported that adoption of sustainable practices in Cameron Highlands in Malaysia is apparently less prompted by extension workers' efforts. They concluded that right support could be identified and extended by government organizations to encourage sustainable agricultural practices faster. According to Alonge and

Martin (1995) the first step toward adoption of new practices by farm producers is to offer relevant information for them which is the main responsibility of extension workers. Fazio et al, (2011) listed categories of extension workers' perceived obstacle to adoption of sustainable practices such as lack of information on sustainable practices. Agunga (1995) reported that extension workers need to be trained in sustainable agriculture in order to expand their knowledge, competence, and ability to communicate the concepts to the farm producers. Furthermore he stated that the logic is clear: if agricultural extension workers are not convinced of the value of sustainable agriculture, how can they be expected to train farm producers?

Extension workers are in a position to promote sustainable agriculture and to facilitate the adoption of sustainable agriculture. Hence they must be competent to adequately orient themselves toward their responsibility on sustainable agriculture. Extension workers in order to promote sustainability practices, they must first understand sustainable agriculture concepts. Thus, the role of extension workers is very crucial in supporting sustainable agriculture (World Bank, 2006; Toness, 2001). According to Karbasioun et al. (2007) and Chizari et al. (2001) low level of extension workers' knowledge and skills with respect to sustainable agriculture is one of the major barriers of adoption of sustainable agriculture activities in Iran. They further concluded that extension workers do not have enough competencies to deliver extension programs regarding environmentally sound agriculture. In addition, Ommani and Chizari (2010) reported that limited farmer knowledge of sustainable agriculture principles and low level of extension workers' knowledge on sustainable agriculture, as some barriers that hinder adoption of sustainable agriculture practices. Results of study conducted by Tiraieyari et al. (2009) revealed that Malaysian extension workers perceived themselves competent in relation to the Good Agricultural Practices as one aspect of sustainable agricultural practices. However the current agricultural practices in Malaysia to some extent vary from the standard of sustainability.

Conclusion

Extension workers play a vital role for success of the sustainable agricultural program. In other words the success of sustainable agricultural program depends to a large extend on training and education of farm producers by extension workers. Researchers have come to the conclusion that extension has not yet convinced most farm producers; hence they must continue to work to convince them. In other words extension workers have not adequately oriented themselves toward their responsibility on sustainable agriculture. One of important challenge for achievement of sustainable agriculture could be insufficiency of skillful extension workers to promote the sustainable agricultural to farm producer. If extension efforts supposed to have an impact, training of extension workers on sustainable development should be the first step. The training programs should cover ecological, social and environmental sustainability. The major roles of agricultural extension workers are transferring information from local research to farm producers in helping them to make decisions. Hence during the process of developing education programs for delivery of information on sustainable agriculture, taking training needs of extension workers should be importantly considered. Adequate number of well-trained extension workers on sustainable agriculture will play significant role to support and improve sustainable agriculture. Therefore a high priority should be given to in-service training programs for agricultural extension workers regarding sustainability issues.

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Ginger Administration has a Protective Effect on the Liver of Albino Rats Treated with 6-Mercaptopurine Drug

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Abstract: The leukemia is considered to be one of the most frequent tumors in childhood. The first line of its treatment is 6-mercaptopurine (6-MP). There are many recorded cytotoxic effects for 6-mercaptopurine on tissues such as liver, pancreas and bone marrow. Nowadays the needs for administration of cytotoxic drugs are increased as the incidence of malignancies increases. So we have to search for a safe method that can minimize the side effects of these cytotoxic drugs. The ginger has many benefits as anti-inflammatory and anti-oxidant substance. Some indicated as a protective effect for ginger on the radiotherapy treated rats and also a protection from the hepatotoxic and gonado-toxic drugs were recorded. This work amid to investigate the protective effects of ginger on liver of rats treated by cytotoxic drug (6-mercaptopurine), a drug of choice in maintenance therapy for leukemia in adult and children. Our hope is to minimize the side effects of the cytotoxic drugs with simple and available procedure. ***Material*** The current study had been carried on 40 white albino rats that divided into four equal groups. Group I: was served as control group. Group II: was given ginger extract only. Group III: was given 6- MP. Group IV: was given 6-MP + ginger extract. The following parameters were examined in all groups: 1- The serum Alanine aminotransferase (ALT), and aspirate aminotransferase (AST). 2- The cell morphology and histo-pathological changes in the liver. ***The results*** of the present study showed that treatment with 6-MP alone caused marked signs of cellular degeneration and necrosis of the hepatic tissues, significant increase in the serum levels of ALT ($P<0.001$) and AST ($P<0.001$) enzymes when compared with control group. However, the treatment by ginger along with 6-MP showed marked regeneration and improvement in the hepatic tissues and significant decrease in the serum levels of liver enzymes; ALT ($P<0.001$) and AST ($P<0.001$) when compared with 6-MP treated group. ***Conclusion:*** These findings indicated that ginger has protective effect against 6-MP induced hepatic toxicity.
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Keywords: Ginger, 6-MP, hepatic toxicity.

1. Introduction:

The leukemia is considered to be one of the most frequent tumors in children [1]. The first line of its treatment is 6-mercaptopurine [2]. There are many recorded cytotoxic effect for 6-mercaptopurine on tissues such as liver, pancreas, and bone marrow, and kidney [3,4,5]

6-Mercaptopurine (6-MP) is a purine anti-metabolite widely used in the treatment of acute lymphoblastic leukemia (ALL) [2]. MP is an inactive pro-drug that requires intracellular activation catalyzed by multiple enzymes to exert cytotoxicity. [6,7] Cytotoxic effects of MP are achieved primarily through the incorporation of TGN (thioguanine nucleotides) into RNA and DNA.[8,9] The incorporation of TGN, as deoxy-TGN triphosphate, inhibits the function of several enzymes involved in DNA replication and repair and induces DNA damage.[10,11]

The usual toxic effects of 6- MP are those of bone marrow depression, nausea, vomiting and diarrhea.[12] Animal studies indicated degenerative changes in the intestinal epithelium and liver which were characterized by impaired function, parenchymal necrosis, cirrhosis, jaundice and frequently severe diarrhea.[3,4]

Samuel and John[12] demonstrated that by the histological examination of the liver after 6-MP treatment, there was a pronounced stasis of bile as indicated by numerous bile thrombi. A prominent disruption of the normal hepatic architecture in the form of a complete loss of the hepatic cords is seen. Many individual hepatic cells are swollen by a metachromatic granular cytoplasm. Many of these cells are multinucleated. There is considerable variation in the size and chromatin pattern of the nuclei. Bile pigment is prominent in the Kupffer cell.

Moreover, Cuffari et al.[5] indicated that hepatitis, pancreatitis, and marrow suppression were generally associated with increased 6-MP levels. In addition Dubinsky et al.[13] have demonstrated drug-induced hepatotoxicity in 10–15% of pediatric patients which has been associated with the 6-MP metabolite and 6-methylmercaptopurine ribonucleotide. Other study demonstrated that the levels of 6-MP and its methylated

metabolites are correlated with the degree of hepatotoxicity during 6-MP maintenance therapy.[14]

Ginger has a long tradition of being very effective in alleviating symptoms of gastrointestinal distress and reducing intestinal gas. Reviews such as Borelli et al.[15] of six double-blind, randomized controlled trials with a total of 675 participants, have demonstrated that Ginger can be as effective as over the counter drugs for the control of nausea in pregnant women.

Modern researches have revealed that ginger possesses numerous therapeutic properties including immune boosting, antioxidant effects, [16,17] an ability to inhibit the formation of inflammatory compounds, and direct anti-inflammatory effects.[18]

Moreover, a number of studies demonstrated ginger root's is effective as a painkiller / anti inflammatory and has a benefits for improving osteoarthritis.[19, 20]

Gingerols, which is the main active components in ginger and the responsible for its distinctive flavor, may also inhibit the growth of human colorectal cancer cells [21] although human studies are yet to confirm that.

Moreover, Rhode et al. [22] results indicated that ginger is a nutraceutical that may have significant therapeutic benefit for ovarian cancer patients as ginger induced cell death in all ovarian cancer cell lines tested.

Jagetia et al.[23] indicated that the pretreatment of mice with ginger, reduced the severity of radiation sickness and the mortality at all doses. The ginger treatment protected mice from GI syndrome as well as bone marrow syndrome. As the pretreatment with ginger reduced the irradiation dose-dependent elevation in the lipid peroxidation and depletion of glutathione. Moreover, Jagetia et al.[24] reports that ginger has a protective effect in the radiotherapy treated rats as it decreased both the severity of radiation sickness and mortality at all the exposure doses through scavenging OH, O2 and ABTS radicals in a dose-dependent manner in vitro.

There are also a recorded protective effects for ginger extract on the hepatotoxic effects of both carbon tetrachloride and acetaminophen as it improved the elevated serum liver enzymes and the protective effect of the extract was confirmed also by histo-pathological examination of the liver.[25]

The aim of the present study was to determine the extent to which ginger, can be used as protective therapies from chemo-toxic effects of 6-MP on the liver cells and its functions.

2. Material and Methods:

Experimental animal:

40 white adult male albino rats (weight 185-200gm) were housed at 21°C–22°C in a 12 hr/12 hr light/dark cycle, fed standard rat chow, and given free access of water. Rats accommodated to the laboratory conditions for 2 weeks before starting the experiment.

Experimental methodology:

The rats were divided into four groups of 10 rats/each. *Group I:* animals were served as control. *Group II:* animal received oral ginger extract at a daily dose of 200 mg/kg body weight by gavage. *Group III:* rats received 6- MP(manufactred by Wellcome Drugs under brand name Puri-nethol) in a dose of 3.5mg/kg/day for 4 weeks. The maintenance therapeutic dose is 2.5 mg/Kg, during the course; dosage may be increased up to 5 mg/Kg [12]. In the present study we used the average dose (3.5mg/kg) orally. *Groups IV:* the ginger with the same dose at group II was gavaged to rats two hours before 6- MP treatment at a same dose with group III.[26]

At the end of the experimental period (6 weeks), blood samples will be collected from each rat through the retro-ocular puncture. For biochemical analysis, sera were obtained by centrifugation of the blood sample and then they were stored at -20Cº until assayed for Alanine aminotransferase (ALT), and aspirate aminotransferase (AST) on the basis of *Gella et al.*[27] Then all studied animals were sacrificed, the livers are excised and will be prepared to histopathological study.

The histopathological preparation:

The liver tissues will be fixed in Bouin's solution for 48 h. Later, they were dehydrated in graded levels of ethanol, cleared in xylene, and embedded in paraffin wax for sectioning. The 5-µm thick sections were cut, mounted on glass slides, and stained with hematoxylin and eosin stain and Masson's trichrome stain for light microscopic analysis[45].

Methods getting aqueous ginger extract:

According to the method of Sakr, [28] the Rhizomes of Z. officinal were shade, dried at room temperature and were crushed to powder. 125 g of the powder were macerated in 1000 ml of distilled water for 12 h. at room temperature and were then filtered to obtain the final aqueous extract. The concentration of the extract is 24 mg/ml.

Statistical analysis:

All analyses were performed using an SPSS program (version 17.0, SPSS, Inc., Chicago, IL). Differences were analyzed via an ANOVA followed by Tukey's post hoc test. Data are presented as means (±S.D) with significance set at $P < 0.05$.

3. Results:

Biochemical analysis:

In the current study it was found that ALT serum level (IU/ml) in control group was 19.9± 1.42 (Mean ± SD).

There were insignificant differences in ALT serum level between the control and ginger treated groups

19.81±1.78 (P>0.05). While, administration of 6-MP significantly increase the ALT serum level 39.27±2.88 when compared with control (P<0.001) and ginger treated group (P<0.001). While administration of ginger along with the 6-MP significantly decrease the levels of ALT 21.47±1.29, when compared with the 6-MP treated group (P<0.001). There were insignificant differences between the ginger+6-MP treated group and both control and ginger treated group (P>0.05) *Table (1); Figure (1).*

The AST serum level (IU/ml) in control group was 41.93± 1.73 (Mean ± SD).There were insignificant differences in AST serum levels between the control and ginger treated groups 43.55±1.96 (P>0.05). While, administration of 6-MP significantly increase the AST serum level 93.43±6.49 when compared with control (P<0.001) and ginger treated group (P<0.001). While administration of ginger along with the 6-MP significantly decrease the levels of AST 47.27±3.66, when compared with the 6-MP treated group (P<0.001). However; There were significant differences in the AST level in ginger+6-MP treated group when compared with both control (p<0.01) and ginger treated group (P<0.05) *Table (2); Figure (2).*

The histopathological examination:

The results of the histopathological study of the livers are going hand in hand with the biochemical results. Light microscopic examination of the transverse sections of livers of the control and ginger treated rats revealed that the livers exhibited normal appearance as it consisted of many hepatic lobules each of which had a central vein and hepatic cords. These hepatic cords were formed by hepatocytes which were irregularly radiating from the central vein towards the periphery and separated from each other by blood sinusoid. The later were lined with Von Kupffer cells which had large fusiform dark nuclei. The hepatocytes are polyhedral in shape with eosinophilic granular cytoplasm and vesicular basophilic nuclei (Fig 3, A &B). Also, the portal tracts in the control and ginger treated rats were similar, both contained normal sizes of portal vein, a branch of hepatic artery and a branch of bile duct (Fig. 4, E &F). A normal fibrous tissue distribution could be seen in the portal tracts in the control and ginger treated rats (Fig.5, I & J).

In contrast, the histopathological finding in 6-MP treated rats showed marked signs of cellular degeneration and necrosis in the centriolobular and mid zonal regions. There were loss of organized hepatic cords; most of the hepatocytes appeared edematous, swollen with irregular cytoplasm and karyolitic or pyknotic nucleoli. Also, some hepatocytes showed cytoplasmic vacuolations. These features with the presence of wide spaces in the sinusoid and in-between the hepatocytes indicated the presence of hydropic degeneration (Fig.3, C).

Moreover, numerous Von kupffer cells were represented with presence of focal necrotic lesion and inflammatory cells infiltration (Fig.3, C1).In addition, there were huge enlargement of a portal vein (Fig. 4, G) with marked deposition of collagen fibers around it. Also the collagen fibers were markedly deposited in the portal tract and within the sinusoids (Fig.5, K) indicating preportal fibrosis.

These features were improved in 6-MP +ginger treated group where most of the hepatocytes appeared similar to the control group except the presence of few cells contained pyknotic nuclei. No wide spaces in between the sinusoids. (Fig.3, D). The portal vein appeared slightly dilated (Fig. 4, H), with few collagen fibers deposition around the portal vein and in the portal tract (Fig. 5, L).

Table (1): Serum levels of ALT (IU/ml) in control, ginger treated, 6-MP treated and ginger + 6-MP treated groups

	control	Ginger treated	6-MP treated	Ginger + 6-MP treated
Mean ±SD	19.9± 1.42	19.81±1.78	39.27±2.88	21.47±1.29
F	237.4			
P	<0.001			
LSD		.093 (p> 0.05)	-19.37 (P<0.001)	-1.57 (p >0.05)
			-19.46 (p< 0.001)	-1.66 (P>0.05)
				17.81 (p< 0.001)

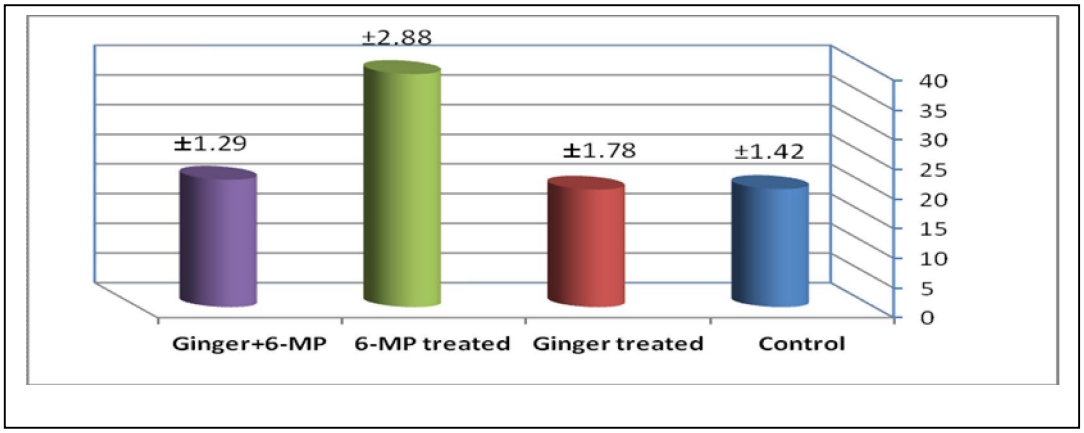

Figure (1): serum levels of ALT (IU/ml) in control, ginger treated, 6-MP treated and ginger + 6-MP treated groups

Table (2): Serum levels of AST (IU/ml) in control, ginger treated, 6-MP treated and ginger + 6-MP treated groups

	control	Ginger treated	DOX treated	Ginger + DOX treated
Mean ±SD	41.93± 1.73	43.55±1.96	93.43±6.49	47.27±3.66
F	391.3			
P	<0.001			
LSD		1.62 (p> 0.05)	51.50 (P<0.001)	5.33 (p < 0.01)
			49.88 (p< 0.001)	3.72 (P<0.001)
				46.16 (p<0.05)

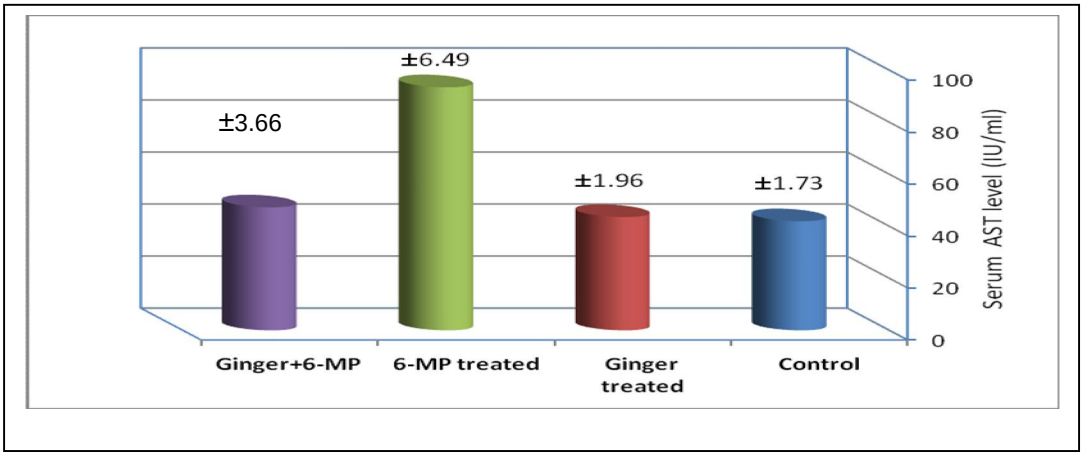

Figure (2): Serum levels of AST (IU/ml) in control, ginger treated, 6-MP treated and ginger + 6-MP treated groups

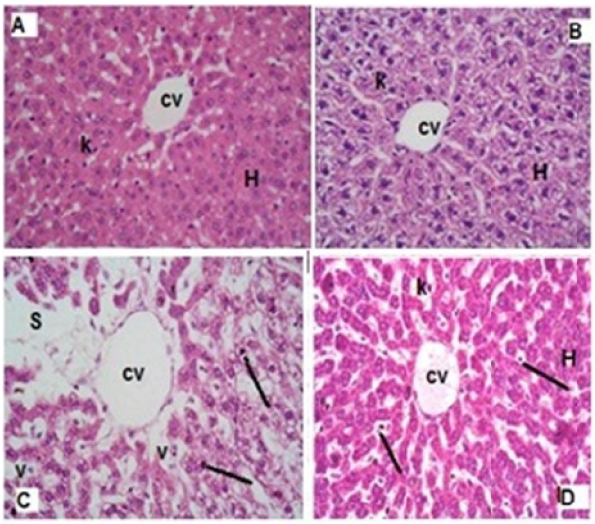

Fig.3. ***Photomicrograph of transverse sections of livers showing:***

(**A**)Control and (**B**) ginger treated group show normal appearance of hepatic architecture, normal central vein (cv), hepatocytes (H) and Von kupffer cells (K). (Hx.& E.; X400)

(C)&(C1) 6MP treated group notice loss of hepatic architecture with presence of wide spaces(S) in-between the hepatic cords, many cells had pyknotic nuclei(/) and others had vacuolated cytoplasm (v) Notice also the presence of cellular inflammatory infiltrations (F). (Hx.& E.;X400)

(D) 6MP+ginger treated group the hepatocytes (H) appeared normal except the presence of few cells had pyknotic nuclei (/) .Notice the presence of normal Von kupffer cells (K) (Hx. &E.; X400)

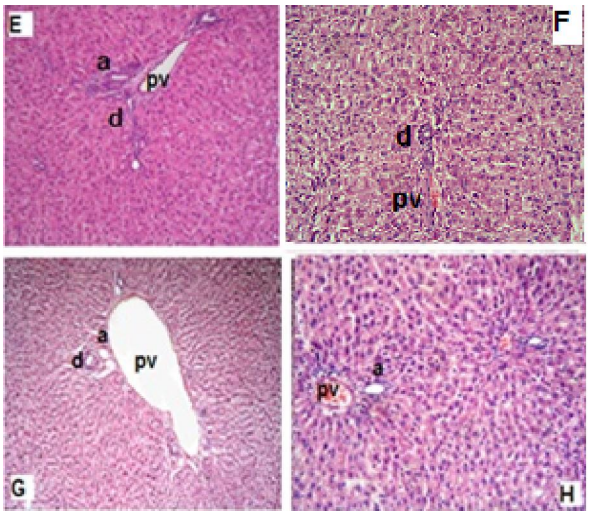

Fig.4**.** ***Photomicrograph of transverse sections of livers demonstrating the portal tracts in:***

(E) Control and **(F)** ginger treated group show normal sizes of portal vein (pv),a branch of hepatic artery (a) and a branch of bile duct (d). (Hx. & E.; X200)

(**G**) 6-MP treated group notice the presence of irregularly dilated portal vein (pv) a branch of hepatic artery (a) and a branch of bile duct (d). (Hx. & E.; X200)

(**H**) 6-MP+ginger treated group the portal (pv) appeared similar to the control and ginger treated group (Hx. & E.; X200).

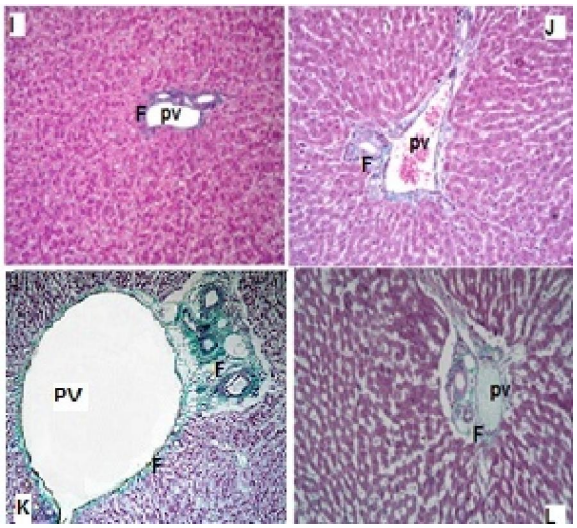

Fig.5. Photomicrograph of transverse sections of livers showing:

(I) Control and **(J)** ginger treated group have normal fibrous tissue (F) distribution around the portal vein (pv) and in the portal tracts (Masson's trichrome X200).

(K) 6-MP treated group notice the presence of hugely dilated portal vein (pv) and marked deposition of collagen fibers (F) around it. Notice also marked deposition of the fibrous tissue in the portal tract and in-between the hepatic cords (F) (Masson's trichrome X100).

(L) 6-MP+ginger treated group show few collagen fibers deposition around the portal vein (pv) and in the portal tract (F) (Masson's trichrome X200).

4. Discussion:

The present study indicated the toxic effect of 6-MP on hepatic tissue and its enzymatic activities. 6-MP was found to cause elevation in serum transaminases (AST, ALT).

That effect was detected by the research of Shorey et al.[29] who concluded that mercaptopurine is generally accepted as potentially hepatotoxic.

The histopathological examination of the liver tissue in the 6-MP treated group revealed marked cytoplasmic vacuolations, deposition of the collagen fibres in the portal tract and within the sinusoids and marked inflammatory cellular infiltration. Bogolipov [30]; Halliwell and Chirico[31] attributed the cytoplamic vaculation to the permeability disorders of the cell membranes caused by excess ROS mediated formation of lipid peroxides. ROS can be generated from the xanthine oxidase (XOD) enzyme which is one of the enzymes that are competing for the initial metabolism of 6-MP.[32]

Moreover, Kumar et al.[33] mentioned that deposition of collagen has lasting consequences on hepatic patterns of blood flow and perfusion of hepatocytes. In the initial stages; fibrosis may develop within or around portal tracts or central vein or may be deposited directly within the sinusoids. Poli and parolia[34] showed the frequent association of the pathogenesis of tissue fibrosis with enhanced lipid peroxidation and deranged antioxidant defence system.

Moreover, the results of the present study confirmed the protective effect of ginger against 6-MP induced liver toxicity, where there was a significant decrease in serum levels of ALT and AST after ginger administration along with 6-MP. In addition, the histopathological features were improved. As ginger administration along with 6-MP improved the dilation of the portal vein, decreased the cellular infiltration and decreased collagen fibres deposition around it and in the portal tract indicating the efficacy of the protective effect of ginger even in the difficult irreversible lesions. These finding goes in line with Kumar et al.[33] who provided a growing evidence for cessation of hepatic injury in some setting can lead to reversal of fibrosis. The best document was regression of fibrosis in treated schistosomal hepatic infection and hereditary hemochromatosis.

Moreover, Sakr et al.[35] found that treating rats with adriamycin and ginger improved the histopathological and biochemical changes induced in the liver by adriamycin. This indicated the effectiveness of ginger in prevention of adriamycin hepato-toxicity.

In addition, Ginger extract was found to have a protective effect on CCl4 and acetaminophen-induced damage as confirmed by histopathological examination of the liver [16]. Siddaraju and Dharmesh [17] reported that ginger-free phenolic and ginger hydrolysed phenolic fractions exhibited free radical scavenging, inhibition of lipid peroxidation, DNA protection and reducing power abilities indicating strong antioxidant properties.

The protective effects of ginger against hepatotoxicity are mainly due to its antioxidant and anti-inflammatory effects. Both water and alcoholic extracts of ginger have been shown to possess potent antioxidant activity on fats and oils and prevent lipid peroxidation, microsomal lipid peroxidation at concentrations greater than 150 mM. In addition zingerone functions as an effective scavenger of superoxide anions as measured by nitro-blue-tetrazolium reduction in a xanthine-xanthine oxidase system [36]. Besides these findings, ginger has antioxidant activity due to its polyphenolic components which have the capacity to be donors of hydrogen atoms or electrons and to capture the free radicals so act as radical scavengers [37]. This is due to the presence of zingeberene and oleoresin [38]. The antioxidant effect of ginger were identified in our pervious study on the protective effect of ginger in the gonadal toxicity caused by Doxorubicin (DOX) as administration of ginger along with the DOX caused significant increase in oxidative stress markers; superoxide desmuatase (SOD) and catalase (CAT) serum levels and reduction in the lipid peroxidation marker; malondialdehyde enzyme (MDA) [39]

Also since the mechanism of hepatic injury may be to due inflammatory process, so the hepato-protective activity of ginger may be due to its content of volatile oils, which showed anti-inflammatory, analgesic and immunomodulatory effects. Volatile oil of ginger is capable of inhibiting T lymphocyte dependent immune reactions [40]. Moreover the anti-inflammatory activity of ginger is due to its ingredients as the gingerols and gingerol analogs (shogaols and paradols). Previous reports have documented the ability of these compounds to directly inhibit prostaglandin and leukotriene synthesis [41]. 6 Gingerol, besides having various pharmacological and physiological effects including anti-inflammatory, analgesic, antipyretic and gastroprotective activities, is also reported to have cardiotonic and anti-hepatotoxic activities [42]. The anti-inflammatory effect of ginger identified in the current study as ginger administration along with the 6-MP significantly decreased the cellular infiltration in hepatic tissues.

Another benefit for ginger administration with chemotherapy may be its chemotherapeutic effect that recently demonstrated by the result of Bode[21] and Zeinab [43, 44]. So, further investigation will be needed to study the chemotherapeutic effect of the ginger.

5. Conclusion

The results of the present work indicated an ameliorative effect of ginger extract against liver damage induced by 6-MP. This effect may be due to its potent antioxidant and anti-inflammatory activities.

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Suitability of Groundwater Quality for Irrigation: A Case Study on Hand Dug Wells in Hantebet Catchment, Tigray, Northern Ethiopia

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Abstract: The study was conducted in Hantebet catchment area which has a total area of 24.5 km^2. The major objective of the study was to assess the suitability of the groundwater quality for irrigation purpose through hydrochemical investigation of the different hand dug wells in the watershed. Having classified the hand dug wells, using the stratified and random sampling techniques, a total of 20 water samples were selected and collected. Water samples were analyzed for alkalinity, sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+}), chloride (Cl^-), bicarbonate (HCO_3^-), carbonate (CO_3^{2-}), sulphate (SO_4^{2-}-S), and nitrate (NO_3^--N) in Water Works Design and Supervision Enterprise Laboratory Service, Addis Ababa. pH and electrical conductivity (EC) were measured in situ. Sodium Adsorption Ratio (SAR) was computed using sodium (Na^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) concentrations in meq/lit. Chemical data of groundwater samples as plotted in trilinear diagram indicated eight water types, Ca-HCO3, Ca-Na-HCO3, Ca-Mg-Na-HCO3, Ca-Na-Mg-HCO3, Na-Ca-HCO3-SO4, Ca-Mg-HCO3-SO4, Ca-Na-Mg-HCO3-SO4 and Ca-Na-HCO3-SO4. Most of the water samples plotted in the Wilcox plot fall in the zone designated C2-S1 and C3-S1 indicating that the groundwater samples generally have low sodium absorption ratio (SAR) and medium to high salinity hazard associated with them. In terms of salinity, eight samples were excellent for irrigation use and twelve samples were with slight to moderate degree of restriction on the basis of ECw. In terms of infiltration, on the basis of ECw and SAR value, eleven samples pose slight to moderate degree of restriction on irrigation. However, nine samples pose none degree of restrictions to its use for irrigation due to its effect on soil infiltration rates. Groundwater in the study area is suitable for surface and sprinkler irrigation use with no chloride toxicity, and with respect to sodium toxicity. Only one sample shows SAR values above 3 (3.095).
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1. Introduction

Irrigation is one of the methods used to increase food production in arid and semi-arid regions. It can enhance food production, promote economic growth and sustainable development, create employment opportunities, and improve living conditions of small-scale farmers and thus contribute to poverty reduction and protects the environment from degradation and pollution (Abraham et al., (2005) cited in Nata et al., (2007),. Furthermore, it increases subsurface water levels and recharge groundwater. On the other hand, if irrigation is not properly managed, it can have adverse effects on environment and public health.

The main economic means of Tigray region is rain fed agriculture. The rainfall is erratic and unreliable. The topography of the area is undulating. Thus with the traditional agricultural practices, natural resources are severely degraded due to human interference as well as natural devastation; the land productivity is declining at alarming rate. As a result, the region is not in a position to cover the annual food requirement of the people. To alleviate the challenges of food insecurity in the region, promotion of irrigated agriculture is given priority in the strategy of the Nation (Mekuria, 2003). As a result hand dug well construction and utilization is practiced in the region as well as by individuals in the processes of food security attaining at the households in sustainable basis. In Hantebet watershed, the households constructed about 154 hand dug wells for irrigation purpose. The households benefited from the intervention by cultivating and producing different high value crops two-three times per annum due to the availability of water. Regardless of its benefit the extension workers as well as beneficiaries do not have any understanding on the suitability of groundwater for irrigation purpose to produce crops and there was no data regarding the suitability of ground water for irrigation purpose in the region, therefore the issue of sustainability related to water quality and quantity has to be addressed early in the process.

Since quality of water is part of the ecological concerns to be considered in the beginning, knowledge of irrigation water is critical to understand what management changes are necessary for long-term productivity (Bohn et al., 1985; Brady, 2002). Besides these, irrigated agricultural crops need very good quality water (FAO, 1985). Therefore, the main purpose of carrying out this research was mainly to assess the groundwater suitability for irrigation in Hantebet watershed of Tigray region.

2. Methodology

2.1. Location

The study area, Hantebet catchment, is located in the southeastern zone of Tigray National Regional State, about 50 km southwest of Mekelle, which is the capital city of Tigray. The catchment is one of the tributary of the Tekeze River, which is a tributary to Atbara. Geographically the study area is located between latitude 13° 16' and 13° 24' N and longitude 39° 12' and 39° 20'E having an area of about 24.5 km^2.

2.2. Data Collection

The water samples were collected in January, 2010 from hand dug wells with the aid of environmental sampler in order to have representative sample free from contamination from sampling tools. After each sample is collected, an insitu measurement was made for conductivity, pH, TDS and temperature using Sension Platinum Series portable pH and Conductivity meter (HACH made). Also measured at the field are coordinates and elevation of each of the locations sampled using GPS. All the water samples were collected in 2 liters plastic bottles which were washed and triple-rinsed with distilled water and with the collection water before sampling and transporting them to the laboratory.

2.3. Sampling

After collecting the EC values at water temperature of all the groundwater in the hand dug wells, it was carefully changed in to the EC at 25°C by using the correction factor, and then all the water points were classified according to their EC values at 25 °C in to five groups adopting the following table (Bauder, et al., 2003).

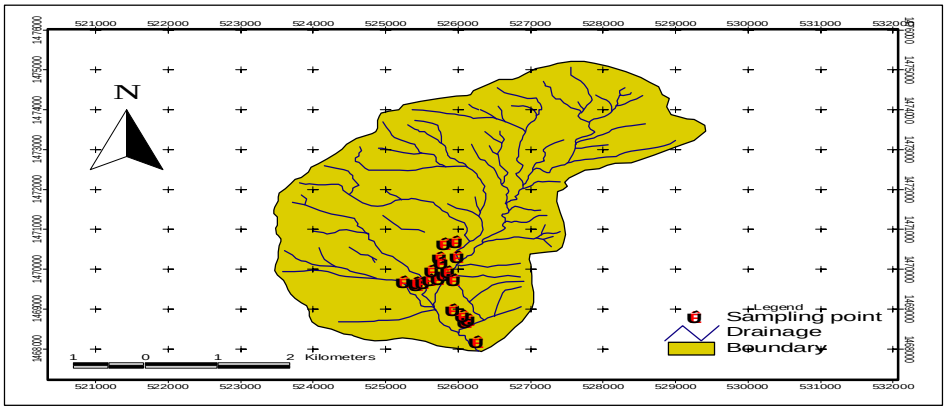

Figure 1: Sampling location of the hand dug wells in the study area

Table 1: Suggested limits for irrigation water use based upon electrical conductivity

S/N	Class	EC(dS/m at 25°C)
1	Excellent	≤ 0.25
2	Good	0.25 – 0.75
3	Permissible[1]	0.76 – 2.00
4	Doubtful[2]	2.01 – 3.00
5	Unsuitable[2]	≥ 3.00

N.B: dS/m at 25°C is equal to mmhos/c, 1 = leaching needed if use, 2 = good drainage needed if used.

Based upon the EC suggested limits for irrigation water use, out of the total 154 hand dug wells, 42 wells were classified to good, 67 wells were classified to be permissible. Since the remaining wells are dried wells, they are not categorized to any one of these classes. Having classified the hand dug wells, using the above stratified classification; random sampling techniques were adopted to take a sample of 20% from each class. Accordingly, 7 samples from good class and 13 from permissible class, a total of 20 water samples were selected and collected approximately with uniform spatial distribution over the study area (Figure 1). The adopted sampling technique was depth integrated sampling.

2.4. Data Analysis

The water samples were analyzed for alkalinity, sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+}), chloride (Cl^-), bicarbonate (HCO_3^-), carbonate (CO_3^{2-}), sulphate (SO_4^{2-}) and nitrate (NO_3^--N) in Water Works Design and Supervision Enterprise Laboratory Service, Addis Ababa.

Sodium Adsorption Ratio (SAR) was computed for each water sample from the analyzed

sodium (Na^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) using the derived calculations stated in (Lloyd, 1985).

$$SAR = \frac{Na^+}{\sqrt{\frac{Ca^{2+} + Mg^{2+}}{2}}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where the concentration of sodium, calcium and magnesium ions is expressed in milliequivalents per liter (meq/lit)

Cations: sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}) and calcium (Ca^{2+}) were analyzed using AAS (Atomic Absorption Spectrophotometer). Anions such as chloride (Cl^-), sulphate (SO_4^{2-}), and nitrate (NO_3^-- N) were analyzed using UV Spectrophotometer. EC meter and pH meter were used to determine the electrical conductivity and pH of each sample. Titration method was used to determine HCO_3^- and CO_3^{2-} ions.

Water chemistry data has been processed using RockWare (2006) Aq•QA, spreadsheet for water analysis. The quality of the irrigation water is assessed in terms of salinity hazard, sodicity hazard, specific ion toxicity and miscellaneous problems. The salinity hazard of the water is expressed by the electrical conductivity (EC_w). The sodicity hazard of the water is determined by calculating the sodium adsorption ratio (SAR) and electrical conductivity of the groundwater. In addition to the two parameters, the quality of the irrigation water is evaluated on specific Cl^- toxicity and Na^+ and miscellaneous problems of NO_3 – N, HCO_3^- and pH. The chemical quality of irrigation waters was assessed by the classification scheme of FAO**,** (1989) stated in Ayers et al. (1994) (Table 2).

3. Results

3.1. Cations and anions

3.1.1. Major Cations

The cationic concentrations in the groundwater samples of the study area were presented in table 4 and figure 2. The respective ranges for Na^+, K^+, Ca^{2+}, and Mg^{2+} in mg/l are 22.5 – 128, 0.2 – 5.3, 75.6 – 117.6 and 4.59 – 33.15, respectively. The lowest and highest concentrations were collected at HAGW-S17 and HAGW-S2 for Na^+, at HAGW-S19 and HAGW-S4 for K^+, at HAGW-S10 and HAGW-S4 for Ca^{2+} and at HAGW-S16 and HAGW-S5 for Mg^{2+}. The mean concentration values for Na^+, K^+, Ca^{2+}, and Mg^{2+} in mg/l were 50.275, 0.975, 94.236 and 17.339, respectively. In the groundwater samples calcium and sodium were dominant cations.

Table 2: Guidelines for interpretation of water quality for irrigation

Potential Irrigation Problem	**Restriction on Use**			
	Units	**None**	**Slight to Moderate**	**Severe**
Salinity				
EC_w (or)	dS/m	<0.7	0.7 - 3.0	>3.0
TDS	mg/l	<450	450 - 2 000	>2 000
Infiltration (**Sodicity**)				
SAR =0-3 and ECw=		>0.7	0.7 - 0.2	<0.2
SAR = 3 - 6 and ECw=		>1.2	1.2 - 0.3	<0.3
SAR= 6 - 12 and ECw=		>1.9	1.9 - 0.5	<0.5
SAR= 12 – 20 and ECw =		>2.9	2.9 - 1.3	<1.3
SAR= 20 - 40 and ECw=		>5.0	5.0 - 2.9	<2.9
Specific Ion Toxicity				
Sodium (Na)				
surface irrigation	SAR	<3	3 - 9	>9
sprinkler irrigation	me/l	<3	>3	
Chloride (Cl)				
surface irrigation	me/l	<4	4 - 10	>10
sprinkler irrigation	me/l	<3	>3	
Boron (B)	mg/l	<0.7	0.7 - 3.0	>3.0
Miscellaneous Effects				
Nitrogen (NO_3-N)	mg/l	<5	5 - 30	>30
Bicarbonate (HCO_3) (overhead sprinkling only)	me/l	<1.5	1.5 - 8.5	>8.5
pH		(Normal Range 6.5 - 8.4)		

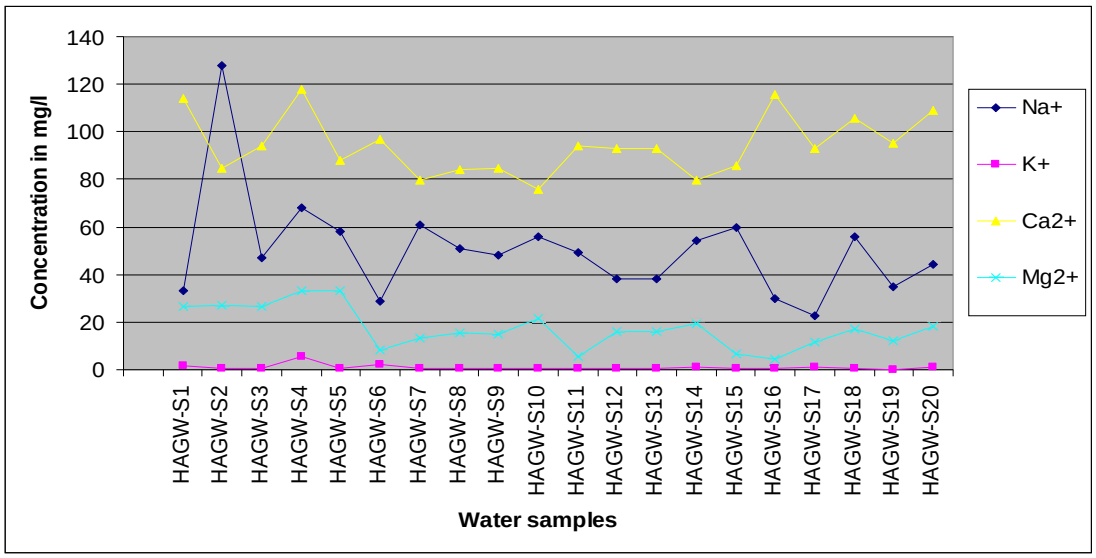

Figure 2: Concentration of major cations in the groundwater sample

3.1.2. Anions

The anionic concentration of Cl^-, SO_4^{2-}, NO_3^- and HCO_3^- in mg/l ranges between 15.45 – 49.44, 16.3 – 148, 0.3 – 5.87 and 312.56 – 589.26 with a mean concentration values of 23.84, 66.22, 1.42 and 420.42, respectively (Table 4 and Figure 3). The anionic concentrations were lowest and highest at HAGW-S8 and HAGW-S4, HAGW-S5 and HAGW-S1, HAGW-S17 and HAGW-S18 and HAGW-S14 and HAGW-S5 for Cl^-, SO_4^{2-}, NO_3^- and HCO_3^-, respectively.

The predominant anions in the study area were bicarbonates and sulphates while carbonates remain nil throughout the groundwater samples (figure 4).

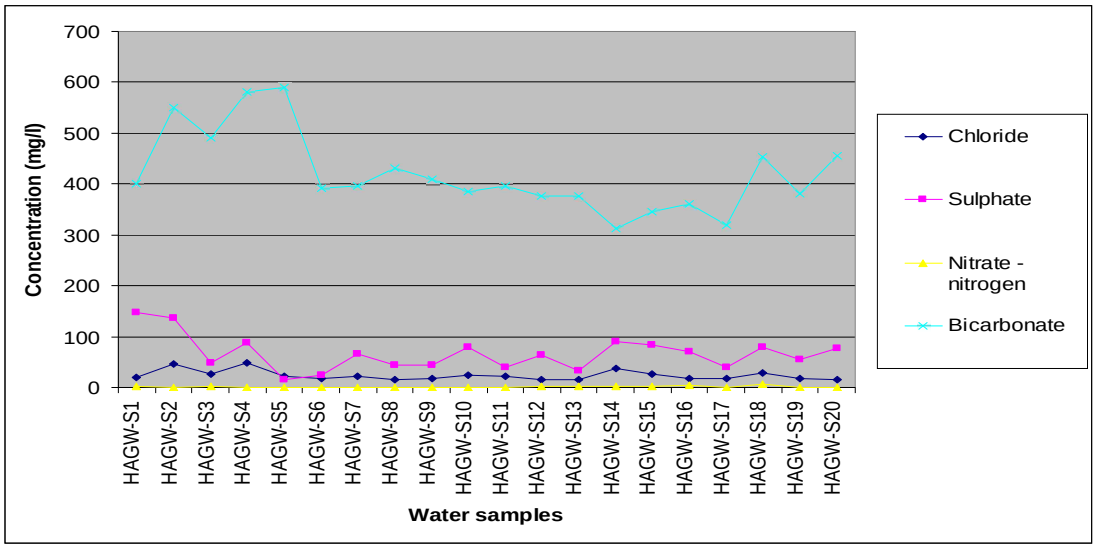

Figure 3: Concentration of anions in the groundwater samples

3.2. ECw, TDS, Alkalinity, pH, Total Hardness and SAR

3.2.1. Electrical Conductivity

The electrical conductivity of the groundwater samples of the hand dug wells used for irrigation ranges from 0.60 dS/m to 1.12 dS/m at 25°C (Table 3). Out of the twenty groundwater samples, six water samples, HAGW-S1, HAGW-S2, HAGW-S3, HAGW-S4, HAGW-S5 and HAGW-S6, had an electrical conductivity value of above 1dS/m with the maximum value of 1.2dS/m obtained at HAGW-S4, while the remaining fourteen water samples,

HAGW-S7, HAGW-S8, HAGW-S9, HAGW-S10, HAGW-S11, HAGW-S12, HAGW-13, HAGW-S14, HAGW-S15, HAGW-S16, HAGW-S17, HAGW-S18, HAGW-S19 and HAGW-S20, had an electrical conductivity value less than 1 dS/m with the minimum value of 0.6 dS/m measured at HAGW-S12.

3.2.2. Total dissolved solids (TDS)

As shown in table 3, TDS values of the groundwater samples of the study area ranges between 300 to 570 mg/l where the lowest value was obtained in the groundwater samples of HAGW-S10 and HAGW-S14 and the highest value was obtained in the groundwater sample HAGW-S5.

3.2.3. Alkalinity

As shown in table 3, the alkalinity of the groundwater samples of the study area ranges from 256.2 to 483 mg/l of $CaCO_3$ where the minimum and the maximum values were observed in HAGW-S14 and HAGW-S5, respectively.

3.2.4. pH

As shown in table 4, the pH value of the groundwater samples of Hantebet basin lied between the ranges of 6.55 to 7.26 at the hand dug wells coded HAGW-S3 and HAGW-S2, respectively.

3.2.5. Total Hardness

Total hardness of the groundwater samples of the study area ranges from 241.5 to 430.5 mg/l of $CaCO_3$ in the HAGW-S15 and HAGW-S4, respectively (Table 3).

3.2.6. Sodium Adsorption Ratio (SAR)

The SAR value of the groundwater samples of the study area ranges from 0.58 to 3.09. The mean SAR value of the groundwater samples of the study area was also found to be 1.25 (Table 3).

Table 3: Computed values of Sodium Adsorption Ratio and measured electrical conductivity (dS/m at 25°C), Alkalinity, Total hardness, Total dissolved solids and Water types for all the analyzed water samples

Sample Code	GPS Location (in UTM)			Alkalinity (mg/l $CaCO_3$)	Total Hardness (mg/l $CaCO_3$)	TDS (mg/l)	ECw at 25°C (dS/m)	SAR	Cl^- (meq/l)	HCO_3^- (meq/l)	Water type
	UTMN	UTME	Elevation (m)								
HAGW-S1	526210	1468122	2198	327.6	394.8	500	1.01	0.722	0.551	6.552	Ca-Mg-HCO3-SO4
HAGW-S2	526046	1468624	2197	451.5	323.4	540	1.02	3.095	1.306	9.030	Na-Ca-HCO3-SO4
HAGW-S3	526085	1468678	2198	403.2	342.3	490	1.08	1.102	0.725	8.064	Ca-Mg-Na-HCO3
HAGW-S4	526016	1468783	2202	476.7	430.5	560	1.12	1.425	1.393	9.534	Ca-Na-Mg-HCO3
HAGW-S5	525877	1468954	2206	483	357	570	1.09	1.335	0.638	9.660	Ca-Mg-Na-HCO3
HAGW-S6	525195	1469634	2212	321.3	275.1	310	1.01	0.760	0.493	6.426	Ca-HCO3
HAGW-S7	525381	1469597	2206	325.5	254.1	400	0.90	1.664	0.609	6.510	Ca-Na-HCO3
HAGW-S8	525459	1469612	2208	352.8	273	390	0.71	1.342	0.435	7.056	Ca-Na-HCO3
HAGW-S9	525557	1469682	2208	336	273	340	0.62	1.263	0.522	6.720	Ca-Na-HCO3
HAGW-S10	525673	1469720	2209	315	277.2	300	0.68	1.463	0.667	6.300	Ca-Na-Mg-HCO3-SO4
HAGW-S11	525753	1469816	2214	325.5	258.3	340	0.66	1.326	0.638	6.510	Ca-Na-HCO3
HAGW-S12	525807	1469894	2213	308.7	298.2	310	0.60	0.957	0.435	6.174	Ca-Na-HCO3
HAGW-S13	525582	1469921	2214	308.7	298.2	330	0.61	0.957	0.435	6.174	Ca-Na-HCO3
HAGW-S14	525719	1470113	2212	256.2	279.3	300	0.68	1.405	1.045	5.124	Ca-Na-HCO3-SO4
HAGW-S15	525700	1470242	2217	283.5	241.5	370	0.76	1.679	0.754	5.670	Ca-Na-HCO3-SO4
HAGW-S16	525761	1470576	2226	296.1	308.7	370	0.68	0.742	0.493	5.922	Ca-HCO3
HAGW-S17	525921	1470646	2222	262.5	281.4	410	0.61	0.583	0.493	5.250	Ca-HCO3
HAGW-S18	525938	1470285	2215	371.7	336	310	0.84	1.328	0.837	7.434	Ca-Na-HCO3
HAGW-S19	525381	1469570	2207	312.9	287.7	350	0.90	0.897	0.522	6.258	Ca-HCO3
HAGW-S20	525891	1469697	2214	373.8	348.6	380	0.76	1.025	0.435	7.476	Ca-Na-HCO3

4. Discussion

4.1. Classification of Groundwater Type

The groundwater types in the area were Ca-Na-HCO3, Ca-HCO3, Ca-Na-HCO3-SO4, Na-Ca-HCO3-SO4, Ca-Na-Mg-HCO3, Ca-Mg-HCO3-SO4, Ca-Na-Mg-HCO3-SO4, and Ca-Mg-Na-HCO3. 40 per cent of the groundwater of the area was Ca-Na-HCO3 (Table 3).

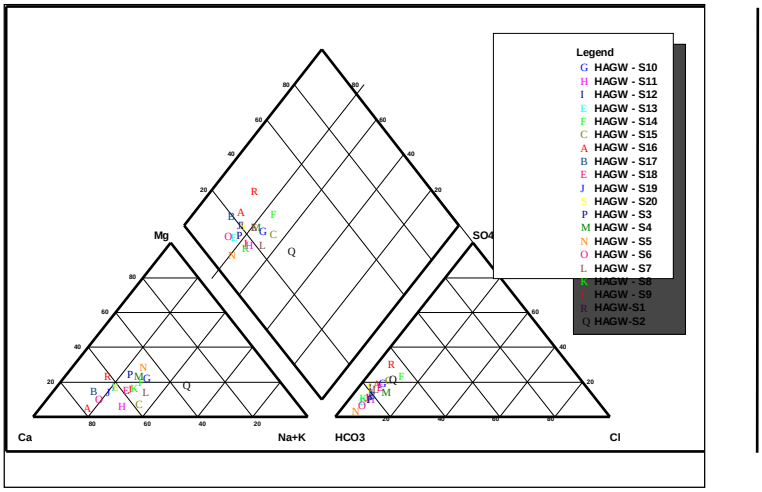

Figure 4: Piper plot of Hantebet groundwater samples

4.2. Suitability of Water for Irrigation Uses

4.2.1. Salinity Hazards

ECw of the groundwater samples of the study area varies widely from 0.6 dS/m to 1.12 dS/m with a mean value of 0.82 dS/m. The greatest and lowest ECw values were obtained at HAGW-S12 and HAGW-S4, respectively (Table 3). Eight groundwater samples are excellent for irrigation use and twelve samples are with slight to moderate degree of restriction since the groundwater samples with < 0.7 dS/m and 0.7 dS/m – 3 dS/m, respectively are none degree of restriction and slight to moderate degree of restriction for irrigation water use (Ayers et al., 1994).

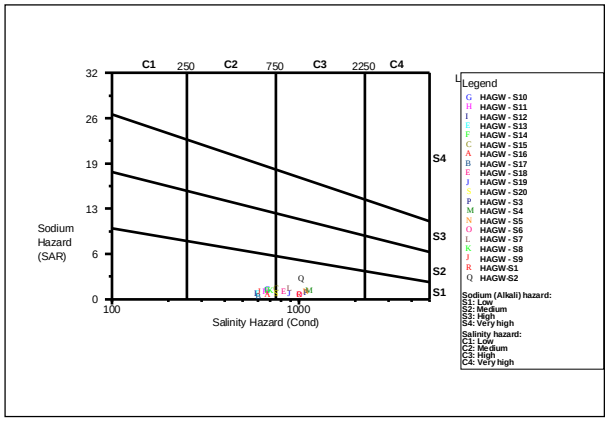

Figure 5: Wilcox plot of Hantebet hand dug well water samples

Furthermore, the Wilcox plot can also be used to quickly determine the viability of water for irrigation purposes Wilcox (1955). The classification of the groundwater of the Hantebet watershed using the Wilcox plot was plotted in the zone designated C2-S1 and C3-S1 indicating that the ground-waters generally have low sodium absorption ratio (SAR) and medium to high salinity hazard associated with them (Figure 5).

4.2.2. Infiltration (Sodicity Hazards)

According to the FAO, (1989) guidelines in table 2, groundwater samples with SAR value 0 – 3 and ECw value of 0.2 – 0.7 dS/m as in the case of eight groundwater samples of the study area (HAGW-S9, HAGW-S10, HAGW-S11, HAGW-S12, HAGW-S13, HAGW-S14, HAGW-S16 and HAGW-S17) and with SAR value 3 – 6 and ECw value of 0.3 – 1.2 dS/m as in two groundwater samples (HAGW-S7 and HAGW-S15) and one groundwater sample (HAGW-S2) with SAR value of 6 – 12 and ECw value of 0.5 – 1.9 dS/m would generally pose slight to moderate degree of restrictions to their use for irrigation due to their effect on soil infiltration rates.

However, nine groundwater samples HAGW-S1, HAGW-S3, HAGW-S4, HAGW-S5, HAGW-S6, HAGW-S8, HAGW-S18, HAGW-S19 and HAGW-S20 have a SAR value between 0 – 3 and ECw value greater than 0.7 dS/m, which pose none degree of restrictions to its use for irrigation due to its effect on soil infiltration rates.

4.2.3. Toxicity Problems

4.2.3.1. Chloride

As shown in table 3, the chloride concentrations in the groundwater samples of the watershed range from 15.45 mg/l to 49.44 mg/l. Though the highest measured chloride 49.44 mg/l occurs in the sample of HAGW-S4 hand dug well, the concentrations of all the groundwater samples are below 4meq/l. This suggests that the groundwater of the study area is suitable for surface and sprinkler irrigation use with no chloride toxicity (Ayers et al., 1994).

4.2.3.2. Sodium

As can be seen from table 4 below, the sodium concentration of the study area ranges from 22.5 mg/l to 128 mg/l in the hand dug wells HAGW-S17 and HAGW-S2, respectively, with a mean value of 50.275 mg/l. The groundwater of the study area is classified into two groups based on their SAR value: nineteen samples have a SAR value of less than 3 and one sample (HAGW-S2) has a SAR value of 3.095, which is greater than 3, with none and slight to moderate degree of restriction of groundwater use for surface irrigation, respectively, based on the FAO, (1989) guidelines stated in Ayers et al., (1994). Nineteen groundwater samples of the study area are suitable for sprinkler irrigation and one groundwater samples (HAGW-S2), which has sodium concentration value of 5.57 meq/l, lies in the slight to moderate degree of restriction for sprinkler irrigation since the water with sodium concentration value of < 3 meq/l has no restriction and > 3 meq/l slight to moderate degree of restriction for sprinkler irrigation (Ayers et al., 1994).

4.2.4. Miscellaneous Problems

4.2.4.1. Bicarbonate

Bicarbonate concentration of the groundwater samples of the study area is greater than 1.5 meq/l. Seventeen have a concentration value of 1.5 – 8.5 meq/l and three groundwater samples (HAGW-S2, HAGW-S4 and HAGW-S5) have a concentration value of greater than 8.5 meq/l (Table 3).

Based on the FAO, (1989) guidelines for irrigation water stated in Ayers et al., (1994), seventeen groundwater samples are with slight to moderate degree of restriction for overhead sprinkler irrigation, however, three groundwater samples are with severe degree of restriction for overhead sprinkler irrigation use because the irrigation water with 1.5 – 8.5 meq/l and > 8.5 meq/l is slight to moderate and severe degree of restriction for overhead sprinkler irrigation (Ayers et al., 1994).

4.2.4.2. Nitrate – nitrogen

Out of the twenty groundwater samples, nineteen groundwater samples had nitrate – nitrogen concentration of < 5 mg/l but only one groundwater sample,HAGW-S18, had a NO_3 – N concentration value of 5.87 mg/l, which was the highest NO_3 – N concentration value in the study area (Table 4). Hence, nineteen groundwater samples were excellent for irrigation but one groundwater sample, HAGW-S18, was with slight to moderate degree of restriction for irrigation.

4.2.4.3. pH

The groundwater of the study area was suitable for irrigation since the pH value lies between the normal ranges of irrigation water given by Ayers et al. (1994) as 6.5 and 8.4 (Table 4).

4.3. Major Cations

4.3.1. Calcium

Irrigation water containing a high proportion of soluble calcium may form scale inside the irrigation component (Pitts et al., 1989) and form scale like deposits on plant parts when overhead sprinkler irrigation system is used (Haman et al., 2000). The highest calcium concentration was observed at HAGW-S4 and value was 117.6 mg/l (5.88 meq/l). The lowest calcium concentration was obtained at HAGW-S10 and its value was 75.6 gm/l (3.780 meq/l) (Table 4). Hence, the groundwater of the study area is suitable for irrigation since the usual range of calcium in irrigation water is 0 – 20 meq/l (Ayers et al. 1994).

4.3.2. Potassium

The minimum and maximum concentration value ranges from 0.2 mg/l to 5.3 mg/l at hand dug wells HAGW-S19 and HAGW-S4. The mean potassium concentration of the study area was found to 0.975 mg/l (Table 5). Hence, nineteen groundwater samples are excellent for long-term irrigation on all soils and crops since the recommended maximum concentrations of K for long-term irrigation use on all soils is 2 mg/l (Ayers et al., 1994 and Duncan et al., 2000). However, only one groundwater sample, HAGW-S4 is normal if used for irrigation since the irrigation water with 5 – 20 mg/l is normal (Duncan et al., 2000).

4.3.3. Magnesium

The concentration of magnesium in the groundwater samples ranges from 4.59 mg/l (0.378 meq/l) to 33.15 mg/l (2.728meq/l) at HAGW-S16 and HAGW-S5 respectively (Table 4). The usual range of magnesium in irrigation water is 0 – 5 meq/l (Ayers et al. 1994). Therefore, the groundwater water of the study area is suitable for irrigation purposes.

Table 4: The major, minor ions, pH and electrical conductivity of water determined in the groundwater samples of Hantebet watershed

Sample Code	Na^+ (mg/l)	K^+ (mg/l)	Ca^{2+} (mg/l)	Mg^{2+} (mg/l)	Cl^- (mg/l)	SO_4^{2-} (mg/l)	NO_3^- (mg/l)	HCO_3^- (mg/l)	CO_3^{2-} (mg/l)	pH	ECw (µS/cm)
HAGW-S1	33	1.7	114.24	26.5	19.57	148	2.1	399.67	0	6.64	1010
HAGW-S2	128	0.6	84.84	27.03	46.35	136	0.97	550.83	0	7.26	1020
HAGW-S3	47	0.7	93.84	26.52	25.75	47.6	2.09	491.9	0	6.55	1080
HAGW-S4	68	5.3	117.6	33.15	49.44	88.6	0.4	581.57	0	6.61	1120
HAGW-S5	58	0.7	88.2	33.15	22.66	16.3	0.4	589.26	0	6.65	1090
HAGW-S6	29	2	96.6	8.16	17.5	23.6	0.49	391.98	0	6.76	1010
HAGW-S7	61	0.5	79.8	13.26	21.63	65.2	0.64	397.11	0	6.83	900
HAGW-S8	51	0.3	84	15.3	15.45	43.6	0.5	430.42	0	6.88	900
HAGW-S9	48	0.5	84.84	14.79	18.54	44.8	0.55	409.92	0	6.94	710
HAGW-S10	56	0.4	75.6	21.42	23.69	80.3	0.75	384.3	0	6.68	620
HAGW-S11	49	0.5	94.08	5.61	22.66	39.39	0.79	397.11	0	6.91	680
HAGW-S12	38	0.8	93.24	15.81	15.45	64.07	1.7	376.61	0	6.86	610
HAGW-S13	38	0.8	93.24	15.81	15.45	32.46	1.51	376.61	0	6.98	660
HAGW-S14	54	1	79.8	19.38	37.08	90.73	1.23	312.56	0	6.90	600
HAGW-S15	60	0.7	85.68	6.63	26.78	83.29	2.61	345.87	0	6.80	760
HAGW-S16	30	0.4	115.92	4.59	17.51	70.07	4.3	361.24	0	6.87	680
HAGW-S17	22.5	0.9	93.24	11.73	17.51	39.13	0.3	320.25	0	6.81	840
HAGW-S18	56	0.4	105.84	17.34	29.7	80.3	5.87	453.47	0	6.79	610
HAGW-S19	35	0.2	94.92	12.24	18.54	54.62	0.88	381.74	0	7.22	680
HAGW-S20	44	1.1	109.2	18.36	15.45	76.25	0.34	456.04	0	7.12	760
Minimum	22.5	0.2	75.6	4.59	15.45	16.3	0.3	312.56	0	6.55	600
Maximum	128	5.3	117.6	33.15	49.44	148	5.87	589.26	0	7.26	1120
Average	50.275	0.975	94.236	17.339	23.836	66.216	1.421	420.423	0.000	6.853	817.000
Standard Deviation	22.040	1.111	12.437	8.499	9.915	33.889	1.441	79.093	0.000	0.190	182.961

5. Conclusions

The results of analysis and interpretation of groundwater samples from Hantebet Watershed for irrigation purpose indicates that samples lie in the slight to moderate degree of restriction and none degree of restriction for irrigation. Generally, groundwater samples of the study area contained desirable levels of concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, NO_3^-, and SAR value, indicating that there would not be any possibility of severe salinity, sodicity, toxicity and miscellaneous problems from irrigation using groundwater. However, Bicarbonate (HCO_3^-) seventeen samples are with 1.5 – 8.5 meq/l and three groundwater samples have a concentration values > 8.5 meq/l which is having severe degree of restriction on irrigation Therefore the groundwater in the study area is fairly suitable for agricultural purposes.

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Direct shoot regeneration from leaf, root and stem internode segments of male poplar trees and the molecular analysis of variant regenerated plants

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Abstract: A regeneration protocol has been developed by using thidiazuron (TDZ) with a high frequency of *in vitro* leaf, root and stem internode induction in male (*Populus alba, Populus tremula* L. and *Populus tremula* L. x *Populus. tremuloides* "Michx"). There were differences in average number of shoots among the different sources of explants, the stem internode explants regenerated shoots more effectively than leaf and root explants. In contrast to root and stem internode explants, leaves had poor regeneration abilities in the case of *P. tremula* and *P. tremula* x *P. tremuloides* "Michx". The highest frequency of adventitious shoot formation was (8.2, 39.2 and 38.3 shoots /explant) for *Populus alba, Populus tremula* L. and *Populus tremula* L. x *Populus tremuloides* "Michx" respectively on a medium containing 0.02 µM TDZ when stem internode explants were cultured. Higher TDZ concentrations significantly stimulated further elongation in the newly formed shoots on the three *Populus* species. *In vitro* regenerated plants were genetically analyzed using RAPD fingerprints. The presence of specific loci in the regenerated plants indicated that no genetic variation existed in the regenerated plants.
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Key words: *In vitro* regeneration, *Populus alba*, *Populus tremula, Populus tremula* x *Populus tremuloides* "Michx", male trees, TDZ, RAPD.

1. Introduction

Poplar (Populus species) are important elements of the riparian ecosystems and target of scientific interest (Tsvetkov et al. 2007). Male clones in some Populus species tended to have longer internodes, higher plant dry weight and heavier wood as compared to the female clones (Khosla and Deol 1984). Recent development in somatic hybridization and direct gene transfer are techniques of interest to extend the genetic variability in Populus. There have been extraordinary developments in molecular genetics and genomics of trees in recent years. These advances were most striking in Populus species and hybrids (Busov et al. 2005) a result of a broad international consensus regarding its value as a scientific and technological model for woody perennial plants (Wullschleger et al. 2002 and Brunner *et al.* 2004). Maximum regeneration rates are the key to successful genetic transformation (Confalonieri *et al.* 2003). Among the possible initial explants root, leaf and stem internode have proven to be a suitable starting point for elaboration of organogenetic systems for *in vitro* regeneration in different species, forest ones inclusive (George 1993**;** Sunpui and Kanchanapoom 2002**;** Shimada *et al.* 1997). *In vitro* cultures of *Populus have* been successfully initiated from several sources of explants including leaf, root and stem (Chaturvedi *et al.* 2004; Tsvetkov *et al.* 2007; Marco *et al.* 2008). This is not the case for male *populus* where this kind of work is limited, especially in aspen and hybrid aspen.

RAPDs are powerful tools for fingerprinting individuals in *Populus* (Kiss *et al.* 2001; Liu and Furnier 1993 and Lu *et al.* 2006). In the present study, RAPD fingerprints from the PCR were used for clonal identification in poplar.

We describe efficient direct shoot regeneration from leaf, root and stem internode explants suitable for genetic transformation, emphasizing the importance of TDZ concentration variation which induced shoot regeneration in the three male poplar species under study. Detection of any probability of somaclonal variations in the *in vitro* regenerated plants was also accomplished.

2. Materials and Methods

2.1 Plant materials

The plant materials used in these experiments were kindly provided by the Institute for Forest Genetics, Grosshansdorf, Germany. It has already been introduced into the culture so that in this paper we only conducted the procedures of regenerated plants from *in vitro* leaf, root and stem internode segments.

Leaf, root and stem internodes [10-15 mm in length (root tips excised) were obtained from stabilized *in vitro* culture of male (*Populus alba, Populus tremula* L. and *Populus tremula* L. x *Populus tremuloides* "Michx"] and used as initial

explants. The explants were distributed separately on Petri dishes (92 x 16 mm) containing 30-35 ml of MS medium (Murashige and Skoog 1962) basic salts and vitamins supplemented with 2.0 % (w/v) sucrose and 6.0 gl^{-1} agar. The pH of the medium was adjusted to 5.7 with NaOH solution (0.1 N) or HCl (0.1 N). The medium was autoclaved for 20 min at 121 C° and different concentrations of thidiazuron (TDZ) (0.0, 0.005, 0.01, 0.02, 0.04 and 0.08 µM) (for root and stem internodes explants) and or (0.0, 0.005, 0.01and 0.02 µM) (for leaf explant), were added to the medium after being autoclaved and cooled to 47 °C. Growth regulator free medium was used as a control. The explants were placed on Petri dishes with adaxial surface in contact with the medium. Every Petri dish was inoculated with six explant discs, each treatment contained ten replicates (Petri dishes). After eight weeks, the average number of regenerated shoots per explant, average length of the longest shoot, explant weight and callus percentage were calculated.

2.2 Genomic DNA extraction

DNA was extracted from fresh leaves of three *in vitro* derived plants which produced from leaf, root and stem internodes separately of the three *populus* species as well as control plants using a standard CTAB extraction procedure (Wolff *et al.* 1994), modified after (Saghai-Maroof *et al.* 1984). Cleaning with ammonium acetate was necessary. Samples were diluted with half the volume of 7.5 M, cold ammonium acetate, cooled in a fridge for 15 min, followed by spinning for 15 min at 5000 rpm. The supernatant was taken and two volumes of cold 96% ethanol gently mixed and left for 30 min in a freezer. After spinning for 15 min, the precipitate was taken, and 500 µl of cold 70 % ethanol was added for washing. The supernatant was removed and the precipitate left to air-dry at room temperature for 10-20 min, and then dissolved in a suitable volume of TE buffer. DNA concentration was determent by NanoDrop 3300 (Thermo Scientific).

2.3 Random Amplified polymorphic DNA (RAPD)

RAPD analysis was performed in 25 µl volume reactions according to Wolff and Peters-Van (1993). A reaction mixture (17.5 ng genomic DNA, 12.5 REDTaq Ready Mix (Sigma) [20 mM Tris-HCl, pH 8.3, 100 mM KCl, 3mM $MgCl_2$, 0.002% gelatin, 0.4 mM mix dNTP(dATP, dCTP, dGTP, dTTP) and 0.06 unit/ µl Taq DNA polymerase] and 0.4 pmole) was prepared for each primer sufficient for all samples plus one negative control in which water was added instead of DNA. All reagents were centrifuged and kept on ice during the preparation of the master mix. Amplifications were carried out in a Mastercycler gradient programmed according to Wolff (1996) [the initial denaturation for 3 min at 94 °C was followed by 45 cycles of denaturation (30 sec. at 94 °C), annealing (45 sec. at 36 °C), extension (1.5 min at 72°C)]. PCR products were analyzed by gel electrophoresis on 1.4% agarose gel prepared in 0.5 X TBE buffers, DNA ladder (Fermentas) was used as a standard with molecular sizes of 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp. The gel was stained with ethidium bromide for 20 min and examined using UV cabinet unit and photographed with a Polaroid camera connected to a computer system with analytical software (GelDocu Advanced version).

Ten different oligonucleotide random primers used for RAPD analysis were:-

(A1) 5'AGACGTCCAC3'
(A2) 5ACGCGCATGT3'
(A3) 5'AATGGCGCAG3'
(A4) 5'GAATCGGCCA3'
(A5) 5'GGGAGACATC3'
(A6) 5'GGAAGTCGCC3'
(A7) 5'ACGCGCATGT3'
(A8) 5'GGTCGGAGAA3'
(A9) 5'CCTACGTCAG3'
(A10) 5' CTGACCAGCC3'

2.4 Statistical analysis

Experiments were set up in completely randomized design. Data were statistically analyzed using ANOVA / MANOVA of Statistica 6 software (Statsoft, 2001), the significance of differences among means was carried out using the Least Significant Test (L.S.D) at $p = 0.05$.

3. Results and Discussion

3.1 Plant regeneration

Regeneration of poplar is the process of micropropagating plants using a small amount of plant tissue and reproducing an entire plant. It is based on the theory of totipotency which states that each cell has everything needed to reproduce an entire plant (Steward, 1968), it is a useful mean of production of plantlets with a lower risk of genetic instability than by other methods. The preliminary experiment using leaf, root and stem internode segments cultured on hormone-free MS basal medium revealed that all explants did not regenerate shoots effectively and became pale and necrotic after 6-8 weeks.

After 1-2 weeks in the culture media contain (0.005, 0.01 and 0.02 µM) TDZ the leaf explants remained green and small shoots were formed only at the margin of the leaf explants Table (1) and Fig (1) in the case of *populus tremula* and *populus tremula* x *populus tremuloides* "Michx". Small granulated callus formed at the margin of the leaves in the case

of *populus alba*, most leaves were induced to form good callus on the MS medium enriched with TDZ. In the beginning, callus growth was rather slow but after 4-weeks culture period the speed of callus formation was fast and small green nodules could be seen. Eight weeks after initial cultures, numerous adventitious shoot formations took place readily from these leaves. In contrast no callus was formed on the leaf margin of *populus tremula* and *populus tremula* x *populus tremuloides* "Michx". **(**Table 1). TDZ promoted production of granular masses of tissues and numerous shoot primordia on the surface of leaf explants of *populus tremula* and *populus tremula* x *populus tremuloides* "Michx". The results showed that TDZ stimulated shoot regeneration and the number of adventitious shoots from leaves explants varied depending on the concentrations of TDZ and the plant species. Considering the positive effect of TDZ on shoot induction, shoot height and explants fresh weight, at optimal TDZ concentration for shoot regeneration from leaf explants were (5.4, 18.2 and 29.1 shoots/ explant) in the case of *populus alba*, *populus tremula* and *populus tremula* x *populus tremuloides* "Michx" respectively. Leaf tissue has been studied and shown to have the greatest regeneration capacity of *Populus* species (Chaturvedi *et al*. 2004; Mingozzi *et al.* 2008).

TDZ at 0.02 µM proved to be the best treatment for direct shoot regeneration since it provide (8.2, 39.2 and 38.3 shoots per explant) from stem internodes of *populus alba*, *populus tremula* and *populus tremula* x *populus tremuloides*, respectively. When TDZ concentration was increased, a reduction in the regeneration capacity was noticed. Stem internodes did not produce shoots on media containing (0.005 and 0.01 µM) TDZ in the three poplar species (Table 2). The highest concentration of TDZ (0.08 µM) induced formation of significantly longer shoots (3.0 cm), with shoot length progressively increasing together with rising of the TDZ concentration in the case of *populus alba* (Table 2). No callus was formed by the stem internode explants in the case of *populus tremula* x *populus tremuloides* "Michx".

The data presented in Table (3) showed that regeneration frequency of root segments as well as callus formation were strongly affected by the thidiazuron concentration in all three species. However, the maximum number of regenerated shoots (5.8, 39.3 and 35.3 shoots/ explant) were obtained when the root segments were cultured on MS medium supplemented with 0.02, 0.01 and 0.02 µM TDZ on *populus alba*, *populus tremula* and *populus tremula* x *populus tremuloides* "Michx", respectively. TDZ was reportedto stimulate shoot organogenesis from root explant of *Populus deltoids* (Chaturvedi *et al.* 2004).

All TDZ- treated cultures yielded healthy shoots in all explant types. The results emphasize the importance of TDZ and suggest that a reasonable TDZ concentration induces shoot regeneration. Thidiazuron (TDZ), a urea-derived cytokinin, is a potent cytokinin for woody plant tissue culture (Huetteman and Preece, 1993) and is extensively used for the induction of shoot regeneration in several plant species (Li *et al.* 2000; Mohan and Krishnamurthy, 2002; Liu *et al.* 2003). It exhibits strong cytokinin-like activity in several bioassays (Mok *et al.* 1982; Mok and Mok, 2001). The inhibition of cytokinin oxidase-mediated degradation of endogenous cytokinins by TDZ is sufficient to account for this extremely high activity. Capelle *et al.* (1983) reported that TDZ stimulated the conversion of cytokinin nucleotides to the biologically more active ribonucleosides. These results indicate that the type of explant is highly important in establishing an efficient regeneration system as reported by Uranbey *et al.* (2005). It could be observed that adventitious shoots frequency of the stem internode explants had regenerated shoots more effectively than leaves and root explants.

3.2 RAPD analysis

Generally, it is important to make sure that the regenerated plants were genetically true-to-type of their donor plants with respect to genetic fidelity for species conservation (Quiala *et al.* 2009). In order to know if there is any aberration in the regenerated plants, the RAPD marker system was employed for this purpose. Three regenerated plants from leaf, root and stem internodes from each species were randomly selected from *in vitro* derived explants as well as control donor plants. Two primers (A6 and A7) of the ten primers tested gave bands in RAPD analysis. The three regenerants and the control shared a large proportion of their RAPD markers (Fig. 4 A and B), which suggests homology among the regenerants and control plant and during our regeneration system no genetic changes had occurred. Results of the present study are in agreement with the results of Lin *et al.* (1993) and Cassells and Curry (2001) that RAPD is useful for establishing a genetic basis for somaclonal variation in poplar. RAPD analysis has been revealed to be a potential marker for distinguishing genetic variation in plant other than poplar (Raimondi *et al.* 2001; S˘us˘ek *et al.* 2002).

Table (1): Effect of different concentrations of TDZ on adventitious shoot regeneration from leaf explants of *Populus alba*, *Populus tremula* and *Populus tremula* x *Populus tremuloides* "Michx"

TDZ (µM)	*P. alba*				*P. tremula*				*P. tremula x P. tremuloides*			
	No. of shoots/ explant	Longest shoot (cm)	Explant fresh weight (g)	Callus %	No. of shoots/ explant	Longest shoot (cm)	Explant fresh weight (g)	Callus %	No. of shoots/ explant	Longest shoot (cm)	Explant fresh weight (g)	Callus %
0.00	0.0b*	0.0b	0.0b	0.0b	0.0b	0.0c	0.0b	0.0	0.0b	0.0b	0.0c	0.0
0.005	3.8a	1.6a	0.5a	70.0a	0.0b	0.0c	0.0b	0.0	0.0b	0.0b	0.0c	0.0
0.01	5.4a	1.7a	0.5a	67.5a	14.7a	1.2b	0.17b	0.0	27.5a	1.8a	0.5b	0.0
0.02	3.9a	2.4a	0.5a	64.3a	18.2a	2.6a	0.69a	0.0	29.1a	2.2a	0.9a	0.0

* Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test

Table (2): Effect of different concentrations of TDZ on adventitious shoot regeneration from internode explants of *Populus alba*, *Populus tremula* and *Populus tremula* x *Populus tremuloides* "Michx"

TDZ (µM)	*P. alba*				*P. tremula*				*P. tremula* x *P. tremuloides*			
	No. of shoots/ explant	Longest shoot (cm))	Explant fresh weight (g)	Callus %	No. of shoots/ explant	Longest shoot (cm)	Explant fresh weight (g)	Callus %	No. of shoots/ explant	Longest shoot (cm)	Explant fresh weight (g)	Callus %
0.00	0.0c*	0.0c	0.0a	0.0b	0.0c	0.0c	0.0c	0.0b	0.0c	0.0b	0.0b	0.0
0.005	0.0c	0.0c	0.0a	0.0b	0.0c	0.0c	0.0c	0.0b	0.0c	0.0b	0.0b	0.0
0.01	0.0c	0.0c	0.0a	0.0b	0.0c	0.0c	0.0c	0.0b	0.0c	0.0b	0.0b	0.0
0.02	8.2a	2.0b	2.5a	50.0a	39.2a	2.3a	1.2a	0.0b	38.3a	1.8a	1.1a	0.0
0.04	2.4b	2.1b	1.2a	100.0a	18.5b	1.5b	1.0ab	70.8a	32.2ab	1.8a	0.89a	0.0
0.08	1.3b	3.0a	2.0a	100.0a	20.8b	1..3b	0.78b	70.0a	28.4b	1.7a	0.72a	0.0

* Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test

Table (3): Effect of different concentrations of TDZ on adventitious shoot regeneration from root explants of *Populus alba*, *Populus tremula* and *Populus tremula* x *Populus tremuloides*"Michx"

TDZ (µM)	*P. alba*				*P. tremula*				*P. tremula* x *P. tremuloides*			
	No. of shoots/ explant	Longest shoot (cm)	Explant fresh weight (g)	Callus %	No. of shoots/ explant	Longest shoot (cm)	Explant fresh weight (g)	Callus %	No. of shoots/ explant	Longest shoot (cm)	Explant fresh weight (g)	Callus %
0.00	0.0c*	0.0c	0.0b	0.0c	0.0d	0.0b	0.0b	0.0c	0.0c	0.0c	0.0b	0.0b
0.005	2.3bc	0.4bc	0.1b	0.0c	26.0b	1.8a	0.4a	0.0c	24.4a	1.7b	0.9ab	0.0b
0.01	3.9ab	1.3b	0.4b	21.4c	39.3a	2.1a	1.1a	0.0c	31.3a	2.6a	1.3ab	0.0b
0.02	5.8a	2.8a	2.1a	75.0b	33.0a	2.5a	1.2a	8.3abc	35.9a	2.8a	1.7a	0.0b
0.04	1.7bc	2.3a	2.2a	100.0a	38.6a	2.3a	1.9a	17.9ab	31.0a	1.7b	1.5a	8.3b
0.08	0.0c	3.0a	3.0a	100.0a	18.0c	2.4a	1.9a	25.0a	26.0a	2.0ab	1.1ab	58.3a

* Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test

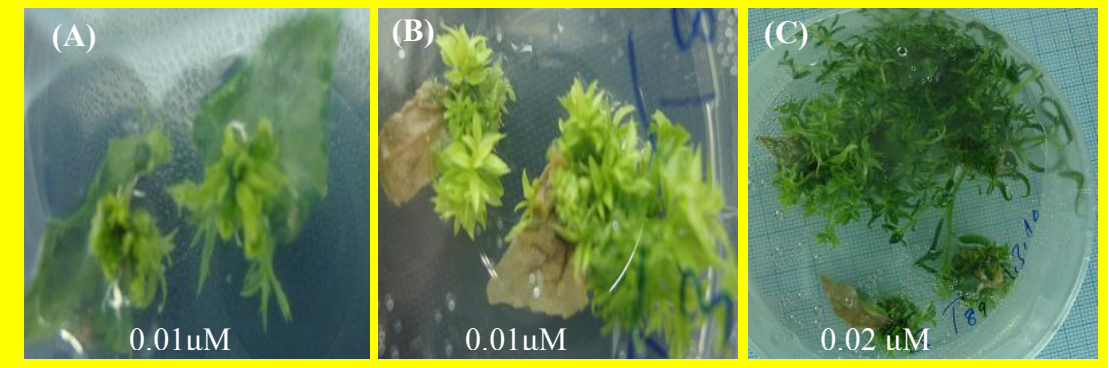

Fig (1): Direct shoot regeneration obtained from leaves on MS medium supplemented with TDZ . (A and B) development of shoot initials on leaves of *P. tremula* x *P. tremuloides* after 4 and 8 weeks in culture, respectively. (C) Adventitious shoots formed on leaves of *P. tremula* x *P. tremuloides* eight weeks after the initial culture.

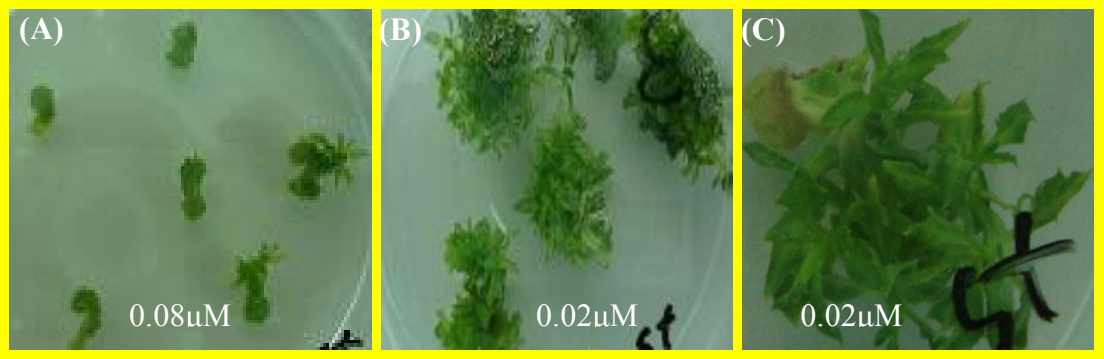

Fig (2): Direct shoot regeneration obtained from stem internode explans on MS medium supplemented with TDZ. (A and B) Adventitious shoots of *P. tremula* x *P. tremuloides* after 4 and 8 weeks in culture, respectively and (C) Adventitious shoots of *P. alba* after eight weeks.

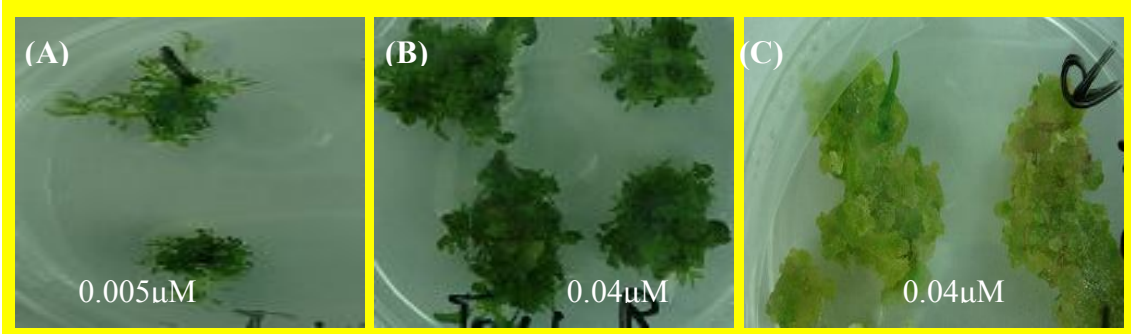

Fig (3): Adventitious shoot regeneration from root explans on MS medium supplemented with TDZ. (A and B) Adventitious shoots of *P. tremula* after 4 and 8 weeks in culture, respectively and (C) Callus formation and development of shoot initials of *P. alba* after eight weeks.

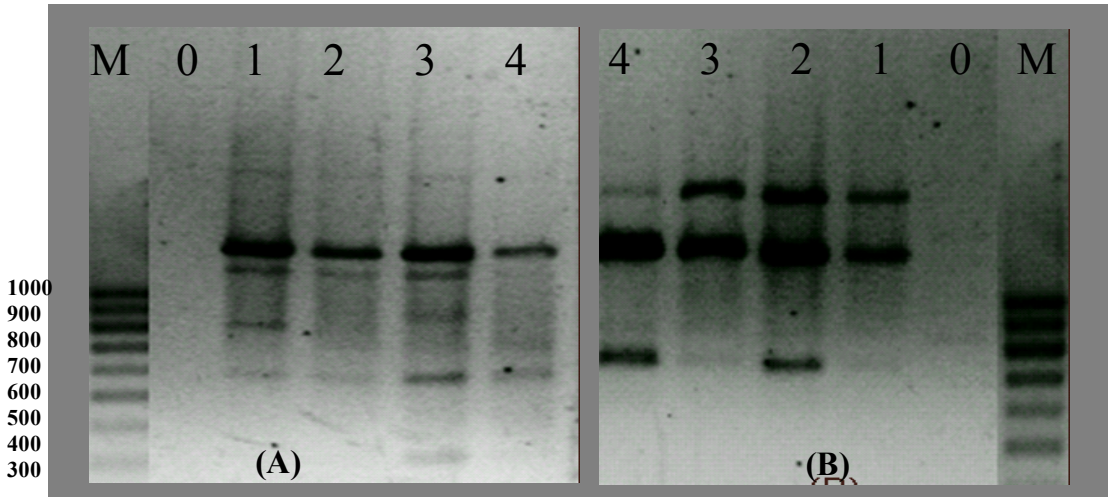

Fig (4): (A and B) Electrophoresis pattern of DNA amplification products obtained after direct shoot regeneration from stem internode and root explans of *P. tremula* x *P. tremuloides* and *P. tremula*, with primers A6 and A7, respectively, Lanes 2, 3 and 4 refer to sample plants, 1 is the control plant, 0 refers to the negative control of PCR and M refers to the DNA marker.

4. Conclusion

Data presented in this study showed a striking improvement of adventitious shoot regeneration in MS medium amended with TDZ which greatly increased adventitious shoot from leaf, stem internode and root explants of different poplar species under study, but regeneration was genotype-dependent. Furthermore, while the same tissue was used in three species, a differentiated response was obtained. There were differences in average number of shoots among the sources of explants in all poplar species. The stem internode explants regenerated shoots more effectively than leaf and root explants. In contrast, leaf had poor regeneration abilities in the case of *populus tremula* and *populus tremula* x *populus tremuloides* "Michx". In conclusion this research demonstrated that direct plant regeneration of the three male poplar trees through culture of leaf, root and stem internode segments could be obtained easily. The rapid clonal propagation described here may be useful for the development of gene transfer system and for micropropagation of genetically stable plants from theses important male trees.

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Assessment of female adolescent Reproductive health needs in Assiut city

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Abstract: Adolescents around 17 million in Egypt (2005) have different needs and require different counseling approaches and more information. Adolescence is a critical stage for risk-taking because adolescents are moving toward independence and tend to experiment and test limits**. Aim of this study**: was to determine the problems of female adolescents, and provide information about reproductive health. **Subject and methods**: Cross-sectional analytic study was conducted in nursing secondary schools and technical institutes in Assiut city in Upper Egypt with total number of 514 adolescent female students which are recruited. The data were collected through a self-administered questionnaire sheet and a physical assessment sheet. **Results**: The study revealed that 94.6% of the girls were circumcised, and 98.2% had their menarche. Overall, 65.5% had satisfactory knowledge, and 81.5% had positive attitude. The main sources of information were classroom whereas parents, newspapers, and magazines were less reported. There was a statistical significance difference between knowledge and had circumcision ($p=0.002$), also, between knowledge about reproduction and their related attitudes and beliefs ($p<0.001$). **Conclusion**: unmet information needs were related to female genital mutilation, family planning, pregnancy and labor, and the preferred sources are school book, media, and parents. **Recommendations:** It is recommended that the unmet needs identified should be used for development of educational programs for adolescents. The role of the parents, as well as health care providers needs to be fostered through using of multimedia as television and radio.
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Key words: Adolescents, reproductive health, knowledge, attitude.

1. Introduction:

Adolescents are inherently different from adults and therefore have different needs because they are less developed physically and psychologically; they have different cognitive abilities and skills, which requires different counseling approaches and takes more time; they tend to be less well informed and therefore require more information; conflicts between culture or parental expectations and the adolescents' emerging values present serious challenges for them; and they are in a transitional state and are not sure where they fit in ***(Carrera, 2006)***.

Adolescence is a critical stage for risk-taking because adolescents are moving toward independence and tend to experiment and test limits, including practicing risky behaviors; substance or drug use typically occurs for the first time during adolescence; sexual experiences (not always voluntary) usually begin during adolescence; consequences of risky behaviors can have serious and long-term effects; adolescents are experiencing pressures from peers, family, society, and the community; and adolescents are easily influenced ***(Eaton et al., 2010)***.

Adolescence is an opportunity time for professional interventions because health education can be integrated into school, home, and religious activities; life-long health habits are established during adolescence; interventions can help adolescents make good decisions and take responsibility for their actions, often preventing serious negative consequences in the future; and there are many effective channels for reaching adolescents such as, through schools, religious institutions, youth organizations, community and recreational activities, parental communication, peer education, the media, and health service facilities ***(Brener et al., 2006; Manlove et al., 2009)***.

Nearly half of the world population and 63 per cent in the least developed countries are currently below age 25, meaning that a large contingent of people will be entering reproductive life in the near future. The population classified as youth, between ages 15 and 24, is estimated to be 1 billion and it comprises nearly 18 per cent of the world population, approximately 14 percent of the population in the more developed regions and nearly 19 per cent of the population in the less developed regions. A large majority of the world's youth lives in less developed regions: 61 percent in Asia, in Egypt around17 million person at age of adolescent***(Brown and Suellentrop, 2009)***.

Very early, adolescents' reproductive rights have been defined as those rights that grant access to

reproductive health information and services, and rights that enable young people to decide freely and responsibly on all aspects of one's sexuality. This includes making informed decisions about sexual expression, relationships, and whether or when to have sex and whether or when to have children. These rights are or should be supported by policies that facilitate access to quality reproductive health information and services for all youth ***(Kelly and Schwartz, 2007)***.

The influence of the family environment on young people's onset of sexual activity is also increasingly acknowledged ***(Munthali et al., 2006)***. Parents and other family members usually play a central role in shaping youth's knowledge, values and attitudes, including those related to sexual and reproductive health. Several studies have documented the influential role of family stability, father's presence in the household and parent-teen communication on the timing of sexual initiation and the reductin of risk-taking behavior ***(Räsänen, 2009)***.

Aim of the study:

The aim of the present study was to determine the problems of female adolescents, and to provide information about reproductive health among them.

Subjects and Methods

Research design& Setting

A cross-sectional analytic research design was used in carrying out the study. It was conducted in nursing secondary schools and technical institutes in Assiut city in Upper Egypt.

Subjectsand Sample size:

The subjects of this study consisted of 514 female adolescents in nursing secondary schools and technical institutes in Assiut city.

The sample size was estimated to determine an expected satisfactory knowledge rate among adolescent girls of 50% or more, with 5% absolute precision and 95% level of confidence. Using the single proportion equation for dichotomous variables:

$$n = \frac{(z_{\alpha/2})^2 p(1-p)}{D^2}$$

Where:

n = sample size

p = 0.50

D = 0.50 x10% = 0.05

The estimated sample size is 384 subjects. After adjustment for a dropout rate of 25%, the sample size was increased to 514.

Fieldwork

Data collection was carried out over a period of four months, from February 2007 to May 2007. After obtaining official approvals, the researcher visited the study settings and met with the headmasters to explain the purpose and procedures of the study. This was followed by recruitment of students from various school grades. The researcher met with them and explained the purpose of the study, the components of the tool, and how to answer the questions. Filling the questionnaire took from 25 to 30 minutes for each student. The researcher was present to answer any questions.

Data collection tool

The data of this study were collected using a self-administered questionnaire sheet and a physical assessment sheet.

Self-administered questionnaire developed by the researcher based on review of pertinent data. The tool was reviewed by nursing and medical experts in obstetrics and gynecology reproductive health, as well as community health. The finalized tool included the following parts:

1st part: Socio-demographic 2nd part : history of circumcision, age, and associated complications, 3rd part: Menstrual history, age at menarche, and 4th part: Knowledge about reproductive health. After completion of the questionnaire, each student was examined using the physical assessment form. Weight and height and pelvic girth were measured according to standard methods ***(Larsson et al., 1984, Himes and Dietz, 1994)***. This was followed by assessment of the vital signs. A special room was prepared in the school for physical assessment by the researcher in special room in order to ensure privacy.

Scoring:

For the knowledge items, a correct response was scored 1 and the incorrect zero. For each area of knowledge, the scores of the items were summed-up and the total divided by the number of the items, giving a mean score for the part. These scores were converted into a percent score. Knowledge was considered satisfactory if the percent score was 60% or more and unsatisfactory if less than 60%.

Physical assessment sheet: developed by the researcher to record the physical examination findings. These included:

Anthropometric measurements: weight and height to calculate to body mass index (BMI) as kg/m^2 height, and pelvic girth by tape measure

Vital signs: pulse, respiration, blood pressure.

Pilot study

After development and validation of the tools, a pilot study was conducted on 50 adolescent girls representing about 10% of the sample size. Its purpose was to test the feasibility of the tools, and to identify any problems related to sequence and clarity of the questions. It also helped the researcher to estimate the time needed to fill the forms. The pilot study findings showed that the questions were clear and relevant, but some wording was modified to increase clarity. The tools were finalized and made ready for use based on the pilot study findings.

Ethical considerations

All principles of ethics in research were followed in carrying out the study. The study protocol was approved by pertinent committees. The purpose of the study was explained to participants, and their written consent to participate in the study was obtained. They were informed about their right to refuse participation, or to withdraw at any time with no reason to be given. They were assured that the information would be totally confidential and used for research purpose only. The questionnaire forms were anonymous. Professional help was provided in case of need. if any of them had a problem referral are done.

Limitations of the study

Some students were reluctant to participate for fear that the questionnaire would affect their academic evaluation. They were convinced about confidentiality, and the forms were anonymous. Others were embarrassed of having the physical assessment, but securing a private place for examination solved the problem.

IV. Statistical Design

Data entry and statistical analysis were done using SPSS 14.0 statistical software package. Data were presented using descriptive statistics in the form of frequencies and percentages for qualitative variables, and means and standard deviations for quantitative variables. Qualitative categorical variables were compared using chi-square test. Whenever the expected values in one or more of the cells in a 2x2 tables was less than 5, Fisher exact test was used instead. Statistical significance was considered at p-value <0.05.

3. Results:

Table (1): describes the socio-demographic characteristics of adolescent girls in the study sample. Their mean age was 16.2±1.1 years, and the highest percentage was in the age category 16 to <17 years (34.0%). Slightly more than half of them (54.3%) had urban residence. The highest percentage was in first and second secondary years (37.9% and 36.2%, respectively). The majority mentioned that the curriculum covered information about reproduction (92.2%). Regarding parents' socio-demographic characteristics shows that about one-fifth of the fathers (20.6%) and slightly less than half of the mothers (47.7%) were illiterate. Meanwhile, only 5.8% of the fathers and 1.8% of the mothers had university education. About two-thirds of the fathers were manual workers (64.2%), whereas the majority of the mothers were housewives (88.7%).

Table (2): demonstrates that the majority of the studied girls were circumcised (94.6%). The age at circumcision varied between less than one to 17 years, with a mean 8.8±2.7 years. He majority of the circumcised girls reported having had related acute problems (86.0%).and done by physician at clinic

Table (3): shows that almost all studied girls had their menarche (98.2%). The cycle was mostly regular (65.5%), with duration of 28 to 30 days (70.7%), three to four days menstrual period (72.5%), and an average number of pads (78.0%). The majority knew that the age at menarche ranged between 12 and less than 16 years (89.1%). As regards the beliefs related to things to be avoided during menses, the majority mentioned prayer (92.2%), followed by Ramadan fast (72.8%) and sexual intercourse (53.7%). Meanwhile, the majority (94.7%) were bathing during menses. Only less than half of the sample had correct knowledge about the time of ovulation (48.4%). In total, 42.6% of the girls had correct knowledge about menstruation, and slightly less than half of them (49.8%) had related positive attitude.

Figure (1): describes the prevalence of menstrual pain in the study sample. It indicates that more than half of the girls had moderate pain (54.5%).

Figure (2): demonstrates that school book was the main source (85.6%), followed by educational media (15.6%). Only 12.8% mentioned parents as sources of information.

Table (4): illustrates the anthropometric measurements of the studied girls. Concerning BMI, it indicates that the majority (70.2%) were normal. Meanwhile, 11.7% and 3.5% were respectively overweight and obese. Meanwhile, slightly less than half of the sample had their girth 90 to less than 100 cm, with a mean 96.0±12.1 cm.

Table (5): illustrated that the majority reported having seen a related diagram (97.9%), mostly in anatomy books (96.2%). Slightly less than half of the sample (45.9%) has correctly mentioned five female genital organs. However, 3.3% of the girls could not name any organ. The table also shows that the highest percentage of the girls could correctly mention only

one part of the vulva (38.5%), and 23.0% correctly mentioned its four parts. The majority had correct knowledge about the location of the female genitalia (90.1%), and the site of implantation (96.1%). Conversely, only one-third correctly knew the source of the menstrual blood (33.3%), and about two-fifth correctly knew the site of sexual intercourse (41.8%). Overall, slightly less than half of the girls had satisfactory knowledge (48.1%).

Table (6): showed that the majority had positive attitude towards age for marriage (90.3%), and pregnancy spacing (79.8%). For the majority of girls, breastfeeding should be two years (89.1%). Overall, slightly more than three-fourth of them had satisfactory knowledge about marriage (75.3%), and 58.0% had related positive attitudes and beliefs.

Table (7): illustrated that the majority knew that diseases could be transmitted by sexual relations (99.4%). The main complications mentioned were infertility (51.0%), followed by urinary tract infection (48.1%), and vaginal secretions (44.2%). The table also shows that the majority (98.8%) knew about AIDS, its manifestations (74.5%), lack of treatment (87.7%), and possible mother-fetal transmission (97.5%). Also, almost all studied girls agreed upon the importance of premarital counseling (98.4%). In total, slightly more than half of the girls (54.1%) had satisfactory knowledge about STDs, and the majority (98.4%) had related positive attitude.

Table (8): showed the majority had correct knowledge about contraceptive pills (96.1%), IUD (92.8%), and other family planning methods (90.7%). Conversely, only 14.4% of them had correct knowledge about the effectiveness of IUD. In total, about three-fourth of the girls had satisfactory knowledge about family planning (74.1%).

Information needs and preferred sources

Table (9): Concerning studied girls' information needs, Table 19 shows that slightly more than half of them (54.7%) have expressed such need. The needed information was mostly for FGM (38.4%), followed by family planning (7.8%), and pregnancy and labor (6.4%). The lowest needs were related to male genital system (0.7%), and male contraception (0.4%). The preferred sources of information were mainly school book (75.5%), educational media (21.2%), and parents (14.4%).

4. Discussion

The adolescence is a period of preparation for adulthood. During this time several key developmental tasks are undertaken. These include physical and sexual maturation, movement toward social and economic independence, and the development of identity. Behavior patterns established during this process, such as drug use or non-use and sexual risk taking or protection can have a long-lasting positive or negative impact on future health and wellbeing. As a result, it is during this process that providers can have the greatest impact on helping young people establish healthy behaviors (Coyle *et al.*, 2003).

The study was carried out on a sample of adolescent girls with age ranging between 14 and 20 years. This age range covers the middle and late adolescence stages as defined by the NCERT (1999). Accordingly, the mid adolescence stage is between 14 and 15 years, and is characterized by the development of a separate identity from family, and tendency to more relationships with peer groups and other sex. The stage of late adolescence is between 16 and 19 years, and is characterized by full physical development, and clear separate identity. Therefore, these stages are more suitable for assessment of issues related to reproductive health.

Slightly more than half of the girls in the present study were from urban areas. This provides a good mix of a sample representing both urban and rural communities, which have different perceptions regarding reproductive health issues. They were selected from nursing schools and institutes for two main reasons. Firstly, they must have better knowledge about reproductive health issues compared to girls in other educational sectors. In support of this, the great majority of them confirmed that their school curricula covered information regarding reproduction. Secondly, their future career implies that they themselves will have the role of educating adolescents regarding health in general, and reproductive health in particular. Therefore, it was deemed important to assess their related knowledge and beliefs.

The description of the socio-demographic characteristics of the parents of the present study adolescent girls is of great importance in shaping their knowledge and beliefs regarding reproductive health. It was found that one-fifth of the fathers about half of the mothers were illiterate. Also, most of the fathers were manual workers, while the majority of the mothers were housewives. These characteristics indicate a low socio-economic level, which will have its repercussions on the study findings as will be shown.

Table 1. Socio-demographic and educational characteristics of adolescent girls and their parents in the study sample (n=514)

Socio-demographic and educational characteristics	Frequency	Percent
Age (years):		
<16	161	31.3
16-	175	34.0
17-	123	23.9
18+	55	10.7
Range	14-20	
mean±SD	16.2±1.1	
Residence:		
Urban	279	54.3
Rural	235	45.7
Nursing education level:		
1st secondary	195	37.9
2nd secondary	186	36.2
3rd secondary	114	22.2
Technical institute	19	3.7
Number of years of education:		
Range	9-14	
mean±SD	9.9±1.0	
Curriculum covers information about reproduction:		
No	40	7.8
Yes	474	92.2
Father education:		
Illiterate	106	20.6
Read/write & Primary	198	38.5
Preparatory& Secondary	190	35
University	30	5.8
Father job:		
Employee	130	25.3
Manual worker	330	64.2
Unemployed	54	10.5
Mother job:		
Housewife	456	88.7
Working	58	11.3
Mother education:		
Illiterate	245	47.7
Read/write & Primary	168	32.3
Preparatory& Secondary	94	18.3
University	9	1.8

Table 2. History of circumcision (FGM) among adolescent girls in the study sample (n=514).

	Frequency	Percent
Had circumcision:		
No	28	5.4
Yes	486	94.6
Age at circumcision in years (n=486):		
<5	28	5.8
5-	233	47.9
10+	225	46.3
Range	<1-17	
mean±SD	8.8±2.7	
Median	9	
Had acute problems (n=486)	418	86.0
Who did the circumcision	458	89.2
By physician or nurse		5.4
By daya	28	

Table 3. Menstrual history and knowledge, attitude & beliefs about menstruation among adolescent girls n the study sample (n=514)

Menstrual history and knowledge, attitude & beliefs about menstruation	Frequency	Percent
Had menarche:		
No	9	1.8
Yes	505	98.2
Age at menarche (years):		
<12	50	9.7
12-	458	89.1
16+	6	1.2
Regularity:		
Regular	331	65.5
Irregular	174	34.5
Things to be avoided during menses:		
Prayer	474	92.2
Ramadan fast	374	72.8
Sexual intercourse	276	53.7
Physical exercises	48	9.3
Going to church	46	8.9
Bathing during menstruation:	487	94.7
Correct time of ovulation	249	48.4
Knowledge about menstruation:		
Satisfactory (60%+)	219	42.6
Unsatisfactory (<60%)	295	57.4
Beliefs and attitudes related to menstruation:		
Positive (60%+)	256	49.8
Negative (<60%)	258	50.2

Figure 1. Prevalence of menstrual pain in the study sample (n=514).

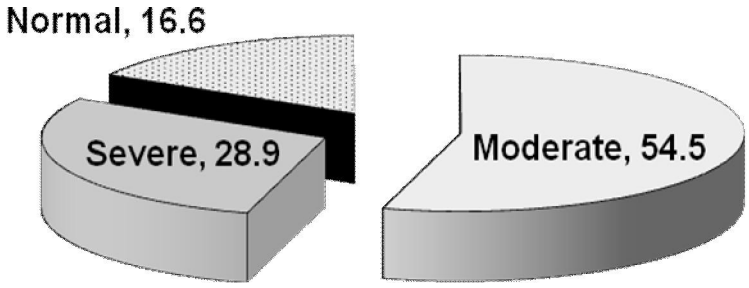

Figure 2. Sources of information about menstruation among adolescent girls in the study sample (n=514)

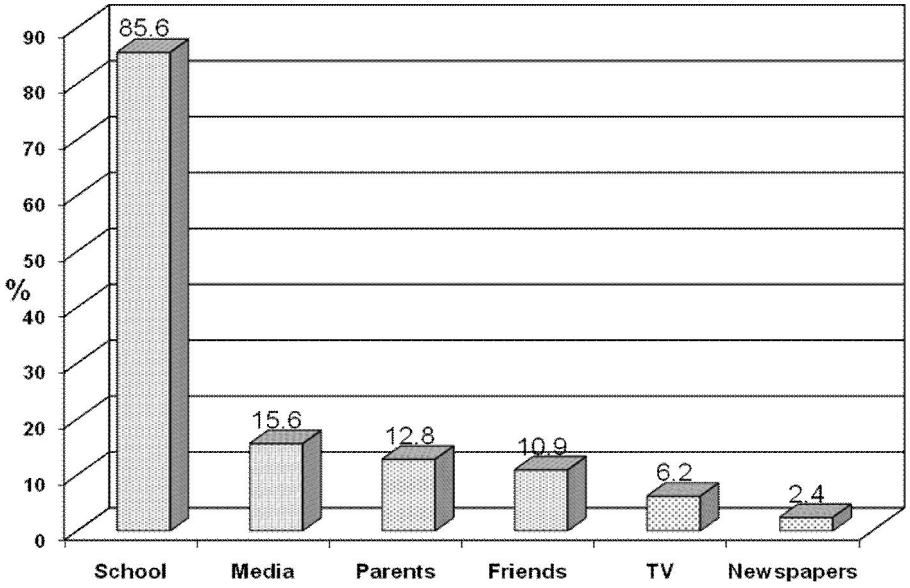

Table 4. Anthropometric measurements of adolescent girls in the study sample (n=514)

Anthropometric measurements	Frequency	Percent
BMI:		
<18.5 (underweight)	75	14.6
18.5- (normal)	361	70.2
25- (overweight)	60	11.7
30+ (obese)	18	3.5
Range	14.0-55.0	
mean±SD	22.1±4.5	
Girth (cm):		
<90	87	16.9
90-	242	47.1
100+	185	36.0
Range	34.0-151.0	
mean±SD	96.0±12.1	

Table 5. Knowledge about anatomy and physiology of female genital system among adolescent girls in the study sample (n=514)

Knowledge about anatomy and physiology of female genital system	Frequency	Percent
Had seen a diagram of female reproductive system:		
Saw diagram in (n=503):		
Anatomy book	484	96.2
School poster	2	0.4
Hospital poster	17	3.4
Correctly know:		
Location of female genitalia	463	90.1
Source of menstrual flow	171	33.3
Site of sexual intercourse	215	41.8
Site of ovum ready for fertilization	250	48.6
Number of fertilizable ova produced monthly	263	51.2
Site of fertilization (ovum and sperm)	262	51.0
Site of implantation	494	96.1
Total knowledge about anatomy/physiology of female system:		
Satisfactory (60%+)	247	48.1
Unsatisfactory (<60%)	267	51.9

Table 6. Knowledge, attitude, and beliefs about marriage and pregnancy among adolescent girls in the study sample (n=514)

Knowledge, attitude, and beliefs about marriage and pregnancy	Frequency	Percent
Appropriate age for marriage (years):		
Range	15-30	
mean±SD	20.3±1.9	
Median	20	
Positive attitude/ correct belief towards:		
Age for marriage	464	90.3
Pain during sexual intercourse	351	68.3
Bleeding in first marital sexual intercourse	380	73.9
Pregnancy spacing	410	79.8
Pain during labor	183	35.6
Duration of breastfeeding (years):		
<2	31	6.0
2	458	89.1
>2	25	4.9
total	458	89.1
Total knowledge about marriage and pregnancy:		
Satisfactory (60%+)	387	75.3
Unsatisfactory (<60%)	127	24.7
Beliefs and attitudes related to marriage and pregnancy:		
Positive (60%+)	298	58.0
Negative (<60%)	216	42.0

Table 7. Knowledge, attitude, and beliefs about sexually transmitted diseases (STDs) among adolescent girls in the study sample (n=514)

Knowledge/attitude/beliefs about sexually transmitted diseases (STDs):	Frequency	Percent
Correctly know:		
Diseases can be transmitted by sexual relations	511	99.4
Complications of STDs:		
Infertility	262	51.0
Urinary tract infection	247	48.1
Vaginal secretions	227	44.2
Death	180	35.0
Urinary secretions	119	23.2
Heart diseases	45	8.8
AIDS	508	98.8
Manifestations of AIDS	383	74.5
Lack of treatment for AIDS	451	87.7
Modes of transmission of AIDS:		
Sexual intercourse	483	94.0
Blood transfusion	392	76.3
Shared syringes	368	71.6
Transmission from mother to fetus	501	97.5
High-risk groups	316	61.5
Importance of pre-marital counseling	506	98.4
Total knowledge about STDs:		
Satisfactory (60%+)	278	54.1
Unsatisfactory (<60%)	236	45.9
Beliefs and attitudes related to STDs:		
Positive (60%+)	506	98.4
Negative (<60%)	8	1.6

Table 8. Knowledge about family planning among adolescent girls in the study sample (n=514)

Knowledge about family planning:	Frequency	Percent
Know about contraceptive pills (OCP)	494	96.1
Know the time of use of OCP	382	74.3
Know about IUD	477	92.8
Know duration of effectiveness of IUD	74	14.4
Site if IUD insertion	407	79.2
Local methods used by men	355	69.1
Other family planning methods	466	90.7
Permanent family planning methods for woman	377	73.3
Permanent family planning methods for men	241	46.9
Knowledge about family planning:		
Satisfactory (60%+)	381	74.1
Unsatisfactory (<60%)	133	25.9

Table 9. Information needs and preferred sources of information about reproductive health as reported by adolescent girls in the study sample (n=514)

	Frequency	Percent
Need more information about reproductive health:		
No	233	45.3
Yes	281	54.7
Information needed (n=281):[@]		
FGM	108	38.4
Family planning	22	7.8
Pregnancy and labor	18	6.4
Menstruation	17	6.0
Adolescence	13	4.6
Sexual intercourse	13	4.6
AIDS	13	4.6
Related religious issues	5	1.8
Premarital counseling	5	1.8
General information	3	1.1
Early marriage	3	1.1
Female genital system	3	1.1
Infertility	3	1.1
Personal hygiene during menses	2	0.7
Male genital system	2	0.7
Male contraception	1	0.4
Preferred sources of information:		
School book	388	75.5
Educational media	109	21.2
Parents	74	14.4
TV	65	12.6
Friends	33	6.4
Magazines	22	4.3
Newspapers	19	3.7

(@) Not mutually exclusive

The very high prevalence of circumcision in the present study is expected given the low socio-economic conditions of their parents, with high illiteracy. Also, about half of them were from rural areas. In line with this, the study of Elnashar and Abdelhady (2007) revealed that 75.8% of the studied sample was circumcised. This unexpected low rate reflects the trend towards rejection of circumcision particularly in some classes. It was attributed to the higher educational levels of the participants, where no illiterates were found among the non-circumcised females and the majority of them were at university level or higher; additionally, the majority was working and living in urban residential areas.

According to the present study findings, the majority of the circumcised girls reported having had related acute problems. This high rate of post-circumcision complications is in line with previous studies that reported serious complications, tragic deaths, and extensive reproductive health problems among young women following this procedure (Larsen and Okonofua, 2002; Yoong *et al.*, 2004; Braddy and Files, 2007).

Almost all adolescent girls in the present study had their menarche. This is quite plausible since the youngest age in the sample was 14 years old, which is beyond the usual age at menarche. Similar age at menarche was previously reported. For example, the average statistically weighted worldwide age at menarche was found to be 13.54 years (Garg *et al.*, 2001). Meanwhile, El-Gilany *et al.* (2005) in Egypt found that the age at menarche ranged from 10-16 years, with a mean and median of 12.9 and 13 years, respectively. This median is much higher compared to the present study, and this might be explained by differences in study settings.

According to adolescent girls' reporting, about one third had irregular cycles, with longer or shorter duration of the cycle, and longer bleeding days, and more than half of them had menstrual pain, mostly of moderate severity. Also, about one-third of them had more or less than average number of pads. This average, as reported by El-Gilany *et al.* (2005) was 2.5 times per day. Concerning the irregularities in time and bleeding, this might be due to that menstruation was at its start among the girls in the

study sample, and this is usually associated with irregularities due to hormonal imbalance. However, Elnashar and Abdelhady (2007) had another explanation. They found that circumcised women had more irregularity of the cycle and more dysmenorrheal, which can result from chronic pelvic infection and is also caused by pelvic congestion. Menstrual flow may be retained due to tiny vaginal opening. Scarring can be the cause of genital infection and disturbances of menstruation.

Concerning physical findings, the majority of the adolescent girls in the present study had normal body mass index (BMI). Meanwhile, about 15% were overweight or obese, and an almost similar percentage was underweight. These findings indicate that obesity is not highly prevalent among them, but on the contrary, they have a problem with underweight. The finding might be explained by the low socio-economic level of their families. In fact, the socioeconomic status has been previously reported to be associated with underweight and obesity (Rennie and Jebb, 2005).

In congruence with the percentages of overweight found in the present study, Gordon-Larsen *et al.* (2004) showed similar levels of incident overweight in the transition from adolescence to young adulthood. The 5-year incidence of overweight in participants in their study ranged from 11.2% in the age category 13-15 years, to 14.6% in those who were 18-20.

However, in disagreement with the present study findings, a study of 9-16-year old rural youth demonstrated higher rates, where 15% of adolescents were chronically obese (Mustillo *et al.*, 2003). The high rate of obesity was attributed to the rapid growth rates that make adolescents vulnerable to excess weight gain, in addition to a variety of social factors that contribute to adolescent obesity including eating and physical activity patterns, especially TV viewing and other types of sedentary physical activity. Therefore, the importance of adolescence as a critical period for development of overweight may depend on the prevalence of many other obesigenic risk factors (Wardle *et al.*, 2006).

On the other hand, lower rates of overweight and obesity, compared to the present study were reported elsewhere. Thus, Vissers *et al.* (2008) demonstrated that in a sample of 16-18-year-old adolescents in Belgium, 9.9% were overweight and 3% were obese. Studies in other European countries also showed low prevalence of overweight and obesity among adolescents (International Association for the Study of Obesity, 2004; Janssen *et al.*, 2005).

As for the sources of information about female and male genital system anatomy and physiology, the great majority of the present study adolescent girls reported that the main source was school. In agreement with this finding, *de Irala et al. (2008)* highlighted that middle and high schools' textbooks are the most common educational resource, making them the backbone of the official curriculum and, to a greater extent, of the culture transmitted in the classroom. However, in Spanish schools, sexual education is a subject that can be covered by any teacher. Sometimes, specific courses about sexuality are given by a school counselor and aimed at parents and/or pupils. However, most education about sexuality is given in biology lessons.

However, in disagreement with the present study results, *Ancheta et al. (2005)* mentioned that most studied adolescent girls reported receiving reproductive health education from both parental (80%) and formal sources (92%). Parents discussed the menstrual cycle more frequently than other sex education topics, while formal sources focused most on teaching about sexually transmitted disease (STDs). The authors also reported that early reproductive health education and education from both parental and formal sources is associated with reduced sexual risk among high-risk adolescent girls.

Concerning knowledge, attitude, and beliefs related to menstruation, the present study revealed that almost a half of the adolescent girls had negative attitudes and related misconceptions. Their knowledge about ovulation and sexual intercourse during menstruation was low. On the other hand, the majority knew the age at menarche, avoided prayer and Ramadan fast, and bathed during menses. Their correct knowledge about age at menarche and religious practices are expected since this is part of their experience and practices. On the other hand, the low knowledge about sexual intercourse during menses is attributed to the conservatism and restrictions on discussing these issues. In agreement with these study findings, *El-Gilany et al. (2005)* reported that the majority of the studied girls were taking showers during menstruation. Also, girls in a study in India had comp8arable findings, and they expressed their need for more information *(Garg et al., 2001).*

Similar to knowledge about the genital system anatomy and physiology, the main source of information about menstruation among adolescent girls in the present study was the school book. The role of the media and parents was minimal in provision of information. This might be attributed to that according to cultural and traditional beliefs in Egypt menstruation is not considered an appropriate topic of discussion, which leads to the lack of accurate and available information among adolescents. Also, in school most young adolescents are probably too embarrassed to ask questions.

Moreover, they might not even have a chance to ask questions.

These present study findings the majority of girls the number of pads changed are available(4-6)pads78%also reported the main source of information about menstruation are school book(85.6)are in disagreement with *El-Gilany et al. (2005)* who found that almost all the girls reported the mass media as their source of knowledge about menstrual hygiene, followed by mothers. In contrast, an earlier study in Alexandria demonstrated that mothers were the most common source of information on the physiology of menstruation and care before and after menarche, with school curricula and the media having a limited role *(El-Shazly et al., 1990).*

The majority of the present study adolescent girls had positive attitude towards age for marriage, pregnancy spacing, and breastfeeding to be two years. In total, more than three-fourth of them had satisfactory knowledge about marriage, and more than half had related positive attitudes and beliefs. The positive attitude towards pregnancy spacing is quite important since closely spaced pregnancies among teenagers will, by definition, increase the risk of repeat teen pregnancies *(Gold et al., 2004).* The optimal interpregnancy interval for preventing adverse birth outcomes appeared to be approximately 18-23 months *(Zhu et al., 1999; 2001; Zhu and Le, 2003)*

Regarding present study adolescent girls' knowledge, attitude, and beliefs related to sexually transmitted diseases (STDs), the findings point to high general knowledge, especially as related to AIDS, its manifestations, lack of treatment, and possible mother-fetal transmission. This high knowledge about AIDS might be explained by the fact that the mass media give great importance to this disease because of its seriousness, but disregard other more common STDs as gonorrhea and herpes. In congruence with this, it was found that a considerable number of adolescents in the U.S. have never heard of chlamydia or gonorrhea, whereas many adolescents have heard of AIDS *(Clark et al., 2002; Zamora et al., 2006).*

Meanwhile, none of their socio-demographic characteristics was significantly related to their knowledge, attitude and beliefs related to reproduction. The only exception was association between satisfactory knowledge and having had circumcision. This might be explained by the experience the circumcised went through during the process of FGM, which might have increased their knowledge about reproductive system.

Furthermore, the present study revealed a statistically significant association between adolescent girls' knowledge about reproduction and their related attitudes and beliefs. It was noticed that more girls with satisfactory knowledge had positive attitudes. The finding indicates the influence of knowledge on attitudes and beliefs, and this should lead to fostering knowledge in order to correct negative attitudes and false beliefs.

The unmet information needs about reproductive health among the present study adolescent girls were mostly related to FGM, family planning, and pregnancy and labor. Similar unmet needs were reported in the study carried out by *Bansah et al. (2007).* They also indicated that prenatal classes needed to be flexible so that if any new issue arose, it could be discussed. Moreover, *Gagnon (2001)* showed that participants wanted to receive specific information that was not necessarily what health-care providers taught, and this was probably due to their cultural values.

Conclusion

The study findings lead to the conclusion that the great majority adolescent nursing students, as other female adolescents in Egypt are circumcised, and had related acute problems. Overweight and obesity are not major problems among them. Only less than two-thirds of them have satisfactory knowledge about reproductive health, although the majority have positive attitude. Knowledge is significantly related to having had circumcision, and also to attitudes and beliefs. Their main sources of information are classroom while parents, health care providers, and media are less reported. The preferred sources of information are school book, educational media, and parents. If this are the findings among nursing students who ought to know better about health-related issues, worse is expected among other adolescents who had no chance to study them.

Recommendations

Based on the study findings, the following recommendations are suggested.

The unmet information needs identified in the study should be the base for development of educational programs for adolescents regarding reproductive health.

Since school was mentioned as the most preferred source of information, this raises the issue of including reproductive health topics in their curricula, not only nursing schools, but also general schools.

The false beliefs identified in relation to menstruation, female genital mutilation, marriage and pregnancy, and home responsibilities must be addressed in health education programs designed for adolescents.

The principles of adult learning with open discussions and active participation of attendants must be followed in any training programs for reproductive health for adolescents.

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Geoelectrical investigation of external corrosion of earth buried pipeline in the coastal area of Gulf Of Guinea

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Abstract: Earth buried transmission pipeline which is coated and protected by impressed current cathodic protection system have been noted to show evidence of external corrosion in its segment buried within low resistivity geomaterials. The resistivity of the geomaterials within the pipeline environments was investigated using electric drilling technique set-up in Schlumberger array. The earth resistivity measured at Ikot Abasi showed low resistivities (12.41-520Ωm), while the geomaterials at Ikot Osuteng produced high resistivity values (1616-15272Ωm). Potential profiling employing close interval potential survey was used to determine extent of the external corrosion as well as the effectiveness of the cathodic protection system. The potential at Ikot Abasi ranges between 331-910mV while that of Ikot Osuteng ranges between 1117-1811mV. The standard practice protective criteria (SP0169) of -850mV showed that the pipeline segment at Ikot Abasi is under severe corrosion while the segment at Ikot Osuteng is well protected by the cathodic protection system.
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Key words: Potential, Protection, Cathodic, Resistivity, Corrosion, Pipeline

Introduction:

Pipeline used for the transportation of petroleum products are usually of steel material (alloy of carbon and iron). The iron content of steel wares off when the pipeline is exposed to hostile subsurface conditions thereby, degrading the pipeline. Corrosion weakens the strength, ductivity and other mechanical properties of a pipeline. This often results in the failure of pipeline when allowed to continue without mitigation. Accompanied with pipeline failure are potential explosion, human and economic risk and environmental disaster (Okoroafor, 2004; Alawode and Ogunleye, 2011). The primary protection for earth buried pipeline against failure due to external corrosion is surface coatings, while cathodic protection systems serve as secondary protection, especially where there are coating holidays (Rajani and Kleiner, 2003; Osakuni and Abam 2004; Koster, 2004; Wansah, 2008 and Evans et al., 2010). The major cause of external corrosion of earthburied pipeline is the soil resistivity which gives a better prediction of soil corrosiveness than any other soil properties (Andrew et al., 2005). Therefore, soil resistivity measurement is imperative in the investigation of external corrosion of buried pipelines.

Soil resistivity can be measured “On line” or “Off line”. The Off line measurements involve collection of core sample within the pipeline environments. The resistivity of the sample is measured in the laboratory using resistivity box (Rim-rukeh and Awatefe, 2006). The “On line” method is referred to as in-situ method; it involves taking surface measurement in the field. The most popular of this method in corrosion investigation are horizontal profiling with Wenner array or vertical profile with Schlumberger array (Ekine and Emujakporue, 2010; Osakuni and Abam 2004 and Evans et al., 2010). A detailed corrosion investigation requires the combination of soil resistivity measurement and electrical potential profiling. The electrical potential profiling uses close interval potential survey (CISP) to scan the pipeline for flaws; this helps in predicting the extent of corrosion as well as the effectiveness of the functioning cathodic protection system installed for the pipeline. CISP involves potential profiling using a saturated $Cu/Cuso_4$ electrode, a high impedance voltmeter, coated copper wire and making electrical contact to the pipeline at test stations spaced along the pipeline route. However, Raouf and Ahmed (2011) reported that pipe-to-soil potential of any earthburied pipeline could equally be obtained segmental along the pipeline route without the need for both the test stations and $Cu/Cuso_4$ half cell. This is by using the new electric concept of pipe-soil-earth system. This method, however has not gained enough confidence in corrosion studies. Crude oil and gas pipelines are considered protected when the minimum potential of –850mV or more negative value measured with Cu/Cuso4 is achieved (SP0169-2007 criteria).

The nature of the local geology and the presence of stray current sources along the pipeline right of way in the area of study were sources of motivation for this study. Stray current has been noted to be a major cause of cathodic protection failure due to

induced stray voltage on the pipeline which later flow off pipelines through low resistivity geomaterials. Induced voltages destabilizes the cathodic protection system allowing the cathodic part of the corrosion cell to behave as anode, thereby causing corrosion on the pipeline in the face of the functioning cathodic protection system that may be installed for the pipeline. This has been a major problem in the petroleum industry. Even though the pipelines have been subjected to impressed current cathodic protection, there is still some degree of corrosion which leads to explosion of the pipelines and eventual environmental degradation. This study demonstrates a geophysical approach for the detection and mitigation of external corrosion of the pipelines in the part of Nigerian sector of the Niger Delta.

The study area is in close proximity to the Jaja creek which opens into the Gulf of Guinea in the Southern part of Nigeria. Geographical coordinates of the study area lies between lat. 4^0 $30^{'}$ N – 4^0 $45^{'}$ N and long.7^0 $30^{'}$ E – 7^0 $42^{'}$ E (Figure. 1). The area is typical of the Niger Delta flood plains with an equatorial climate. The forest in the area is swampy with mangrove trees and experiences two seasons (wet and dry) which are not clearly defined. The wet season is noted for heavy rain fall while dry season do have light showers of rain. This gives rise to seasonal fluctuations in the ground water table in the area. The near surface geology of the study area shows that the sediments are muddy, clayey, silty and lateritic and of medium-coarse and poorly sorted grained sands. The area is regionally underlain by the Quaternary to Tertiary near shore sediments of the Benin Formation (Figure 2).

Materials and Methods:

The flow of electrical current through an electrolyte can be represented by Laplace's equation (Adey and Hang, 1999), given as

$$\rho \nabla^2 E = 0 \qquad (1)$$

where E is the potential gradient and ρ is the resistivity of electrolyte. The introduction of Green's theorem to the Laplace's equation, gives the boundary element method formulation (Brebbia and Dominguez, 1980):

$$\int_{\Sigma} E\left(\nabla^2 E^*\right) d\rho = \int_{\Gamma} \left(q^{\bullet} E - E^* q\right) d\Gamma \qquad (2)$$

where E^* is the weighting function, q is the normal derivative of E, ρ is resistivity of electrolyte, q^* is the normal derivative of E^* and Γ is the surface condition of the pipe given by

$$\Gamma = \Gamma_A + \Gamma_c + \Gamma_1 \qquad (3)$$

where Γ_A is the anode surface, Γ_c is the cathode surface, and Γ_1 is the insulated (coated) surface. Applying variable transformation in equation (2) and introducing boundary elements, we have

$$K_j E_j = \sum_{j-1}^{N} \int_{\Gamma j} \left(E^* q - E q^*\right) d\Gamma_j \qquad (4)$$

where N is the numbers of boundary elements and K is a dimensionless constant.

The matrix form of equation (4) is given by

$$[A]\{E\} = \{J\} \qquad (5)$$

where $[A]$ is the coefficient of the matrix, $\{E\}$ is a vector of unknown values of potential and normal electric field on the boundary, and $\{J\}$ is an independent vector, which represents the current density.

The electrode Kinetics describes the oxidation and reduction processes that take place between the anode and cathode respectively. These processes can be expressed mathematically as non-linear relationship involving the density current and potential on the metallic surfaces (Adey and Hang, 1999) given as:

$$Ja = fa(Ea) \qquad (6)$$

$$Jc = fc(Ec) \qquad (7)$$

where a and c are anode and cathode respectively, J is the current density, f is a function which represents the relationship between the potential and current due to electrode kinetics, and E is the surface electro potential. Equations (6) and (7) are functions of structure and environmental factors. In most cases one of the important factors is the buildup of calcareous deposits on the cathode section of structure (pipe) wherever the structure is polarized sufficiently in saline environment. This deposit is in addition to any organic film and marine growth being formed (Harvey, 1995). The polarization reduces the effective surface area of the structure involved in the corrosion process by introducing an additional physical resistance, which builds up over time. Thus polarization describes not only the electrochemical reduction but also the environmental factors, which can generally be expressed as

$$J = f\left(E, h, v, D, T\right) \qquad (8)$$

where v is the flow velocity of the electrolyte (in this case, it is referred to as transmissivity), D is depth, T is the temperature and h is the film thickness.

Equation (8) can be assembled to form a mathematical model in vector form given by (Adey and Niku, 1992):

$$\vec{J} = \vec{B}(E, h, v.....) \quad (9)$$

where $\vec{B}$ is a coordinate vector.

Applying equation (9) to equation (5) we have

$$AE = B(E, h, v, A.....) \quad (10)$$

Therefore,

$$A - B(E, h, v.....)/E = O \quad (11)$$

Equation (11) is the non-linear system of equations, which can be solved to obtain the potential E and consequently current density J .

The soil electrical resistivity was measured using McOhm terrameter and its accessories. During the field survey all precautionary measures to ensure that the pipeline does not influence measured resistivity were adhered to. One of such precautions was planting of electrodes not closer than 5m to the pipeline right of way (Figure 3). The vertical electrical sounding (VES) technique employing Schlumberger electrode array was used to obtain fourteen (14) sounding using two chosen profiles. The maximum current electrodes spacing used for the investigation was forty (40) meter; this was due to the relative shallow depth of burial of the pipeline (1.8m). For detailed survey, the sounding points were chosen between 20-25m along a profile parallel to pipeline right of way.

The pipe-to-soil potential measurement was carried out using the "On" mode close interval survey at a spacing of 5m intervals. The $Cu/Cuso_4$ electrode was used as a reference electrode (nonpolarizable electrode) and a contact was made with the permanent test points. The potential between the pipeline segment and the soil was recorded from the high impedance voltmeter (Figure 4). Resistivity data analysis started with the conversion of measured resistance to apparent resistivity values, which were then manually plotted against half the current electrode spacing for purposes of manual smoothening. The smoothened data were fed into IPI2Win software developed for forward modeling. The data obtained were iterated using the same software to obtain the final geoelectrical layer parameters.

Results and Discussion

Typical model curves for the study area are presented as Figure 5 and 6. A correlation of the VES data with geology of the area obtained from borehole lithology log shows that the geologic section differs slightly from the geoelectric sections in their thickness. Hence different resistivity values were assigned to the same geologic layer. This is due to the near surface variations in the electrochemical properties of the soil. However, the resistivity range indicates the same geologic material between 1.8 and 9m (Figure 7). Therefore a good correlation was achieved. Figure 8 also shows a good correlation between geology and geoelectrical sections. The pipe-to-soil potential measured shows evidence of corrosion as well as ineffectiveness of the cathodic protection system at Ikot Abasi indicating that the protective potential is more positive than – 850mV (Figure 9). The pipeline segment at Ikot Osuteng produces potential more negative than -850 mV. Therefore, this segment of pipeline is considered to be adequately protected.

The presence of stray current sources periodically play down on the effectiveness of the cathodic protection system which causes current to flow off the pipeline along the path of least earth resistance. The resistivity survey showed that Ikot Abasi is the site for low resistivity. This low resistivity is due to the clay mineral delineated at pipeline depth. Hence it is the site for corrosion hot spot. Whereas the high resistivity values of geomaterials at Ikot Osuteng impedes current from flowing off the pipeline, hence the pipeline segment is maintained as cathode with respect to the environment. The corrosion nature of the pipelines adjacent to the study area had earlier been reported by Ogbonna (2008) using atmospheric corrosion mechanism. The results from this study have shown that the subsurface is also corrosive to buried metals

Conclusion:

This study shows that the pipeline segments in the area of study are well protected at Ikot Osuteng but poorly protected at Ikot Abasi. The presence of stray current sources along the pipeline right of way is believed to destabilize the functioning cathodic protection system leading to current flowing off the pipeline through the low resistivity geomaterials at Ikot Abasi. The geomaterials identified at Ikot Abasi are lateritic and clayey with low resistivity (14.81-37.22 Ωm) within the limit of maximum current penetration. While the geomaterials identified at Ikot Osuteng were medium - coarse grained sands and fine sands with high resistivity values (1132-3710Ωm). These differences in geomaterials as well as their resistivities variation along the pipeline depth invariably made the segment at Ikot Abasi anodic with respect to the cathodic segment at Ikot Osuteng. However, if the effectiveness of the cathodic protection system was not sacrificed by the stray current sources in the pipeline environments, every

segment of the pipeline will remain protected. To mitigate corrosion due to stray current, an earthing system compatible with the functioning cathodic protection system is recommended. The earthing system will complement the cathodic protection system as well as the coating system. This can go a long way to safeguarding the pipeline against external corrosion. Excavation for direct inspection and repairs is also recommended.

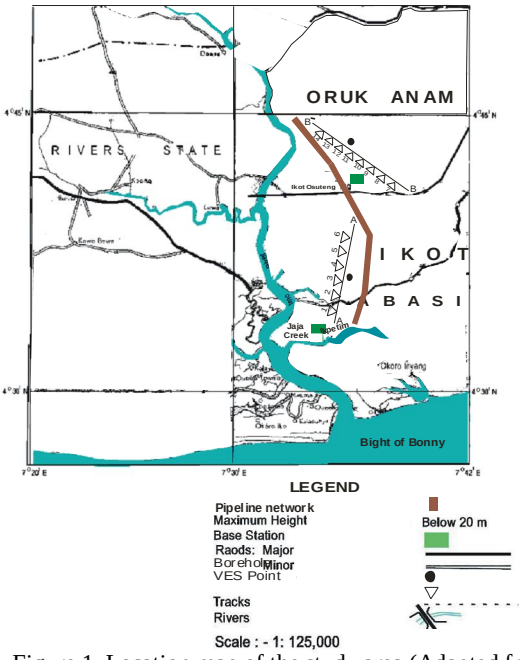

Figure 1. Location map of the study area (Adapted from soil and land use map of Akwa Ibom State, 1989)

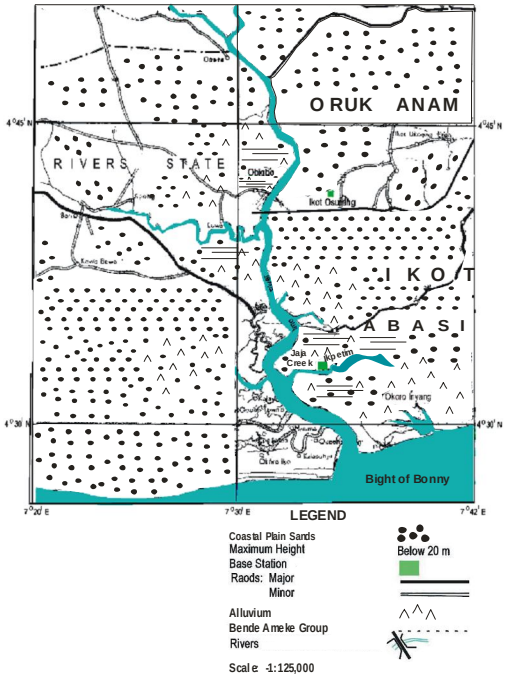

Figure 2. Geological map of the study area (Adapted from soil and land use map of Akwa Ibom State, 1989)

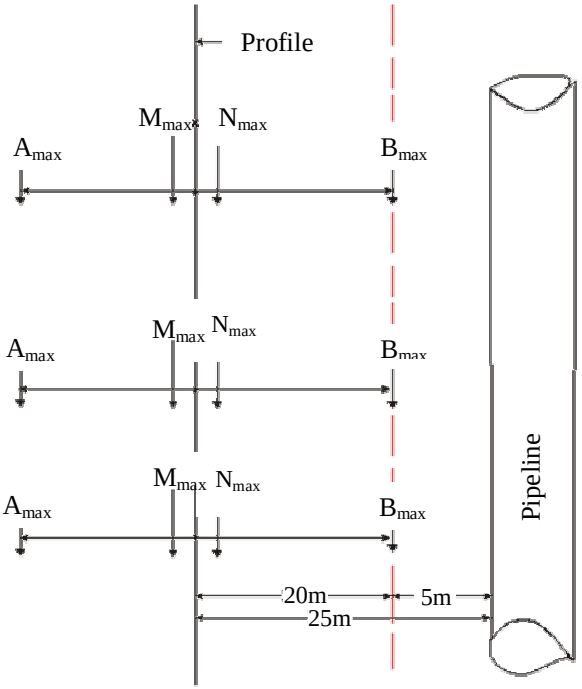

Figure 3. Resistivity measurement within earth buried pipeline system

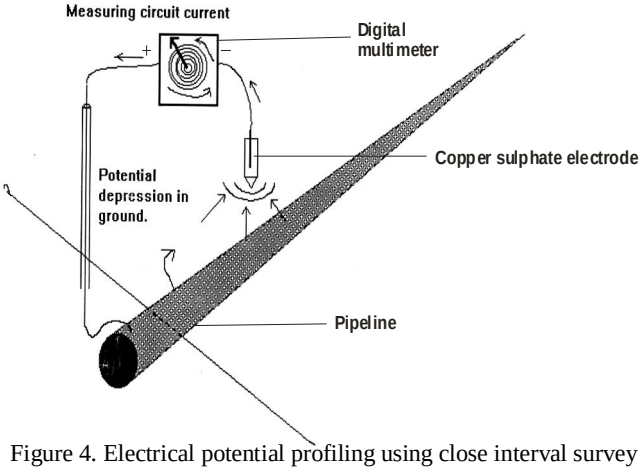

Figure 4. Electrical potential profiling using close interval survey

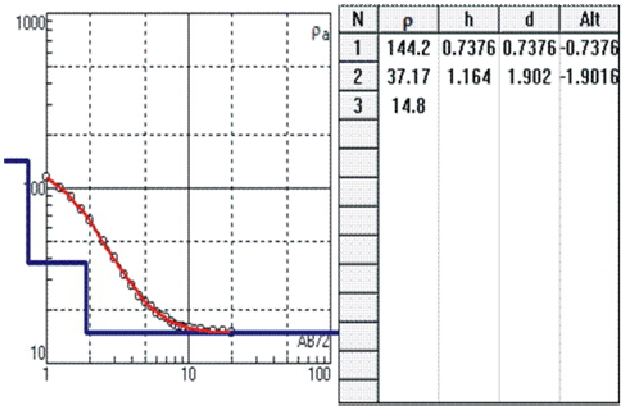

Figure 5. Representative modeled curve for Ikot Abasi

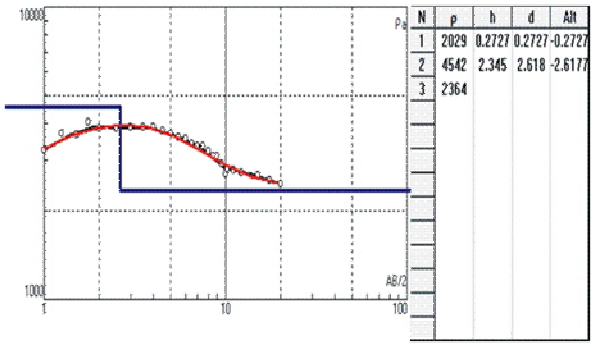

Figure 6. Representative modelled curve for Ikot Osuteng

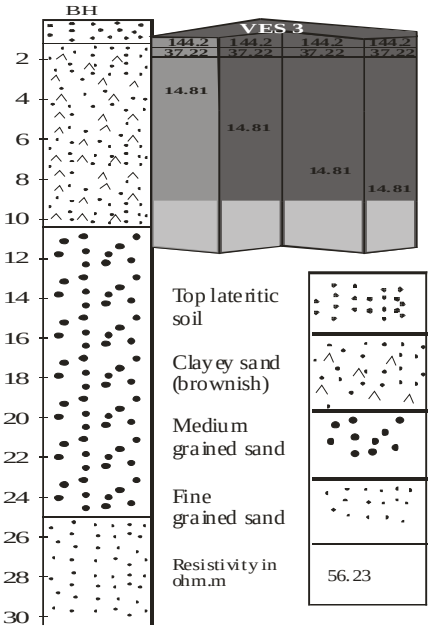

Figure 7. Correlation of VES data with borehole log at Ikot Abasi

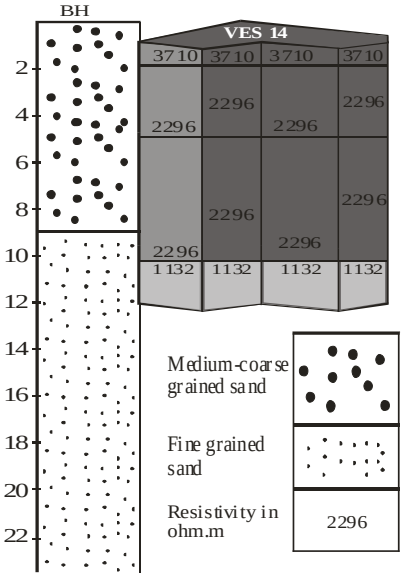

Figure 8. Correlation of VES data with borehole log at Ikot Osuteng

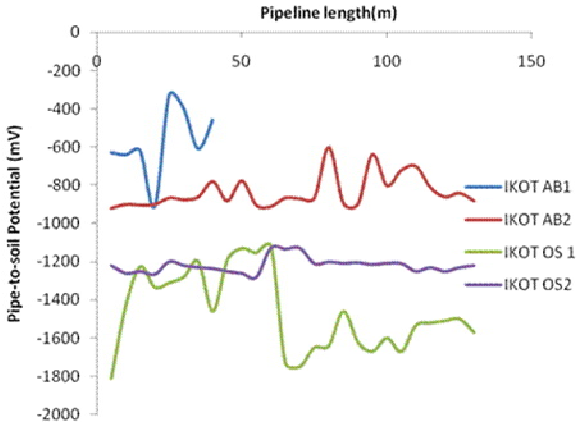

Figure 9. Graph of pipe-to- soil potential against pipeline length

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7/5/2011.

Economic Resource Situation of Future Researching (Income gained by females) in Middle East in 2025 Horizon

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Abstract: From the beginning of 3rd millennium gender indices of strength could be expressive of capability of countries difference in the scale of development. Gender development of strength indices not only pays to rate of political and economical partnership of females, but also embraces the strength of economic resources which is measured by gained income by females and males. Structural change of labor force and entrancing females in various fields of labor and activity because of arising level of females education causes decreasing of gap of their partnership with male cause change of attitude relative to difference of level of incomes and wages between females and male. Achievable observations indicate that there is sexual income gap in all countries and can be very different and even in some cases conversely. However, for example in Brazil, females under 25 years have more average hour wage to their male partners. Since females mostly are working in occupations with low wages, naturally have lower income to the males. Average per capita income by share of wages and non-agriculture incomes for Net national production is lower than mails. But what separates developed and developing countries in the field of income between male and female is the rate of this gap.
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Keywords: Economic Resource, females, developing Countries

1. Introduction

From the beginning of 3rd millennium gender indices of strength could be expressive of capability of countries difference in the scale of development. Gender development of strength indices not only pays to rate of political and economical partnership of females, but also embraces the strength of economic resources which is measured by gained income by females and males.
Structural change of labor force and entrancing females in various fields of labor and activity because of arising level of females education causes decreasing of gap of their partnership with male cause change of attitude relative to difference of level of incomes and wages between females and male.
However, in recent years, presence of females in occupation fields and economic partnership has increased, but females's success in achieving suitable occupational opportunities cannot lonely cause human security and respect, since, females rights in labor market such as equity of incomes has not observed yet.

Non-official activities, lack of supervision and legal support of females's labor is including of existent challenges on the way of achieving females to incomes and wages equal to male. Among wage earner workers and salary earner there are many females who have irregular occupation and since employment of male is more regular than females and male gain more wages in occupations similar to females, females find more desire to border and unsafe occupations such as house works or temporary occupations which has very low wages and with irregular income without occupational security or low income with lack of social support. Even in some countries in female occupations such as nursing and teaching, males receive more wage in comparison with females.

Recent research shows that if females in all occupations which are occupying it, were received suitable salary and equal to males, economics situation of world was very better than present.

Studies in Middle East are forecasting that increasing of females' partnership in work force can increase household income to 25%. Nowadays in Middle East region there are 61 million female in work age that only 17 million of them are occupied and females have less than one forth of non-agriculture works in many countries such as Egypt, Iran, Pakistan and Turkey. Low training level for females in some nations and lack of females achievement to financial credit who cause them problem for their job, including less partnership reasons and following it less income of females in relative to males. In some countries such as Jordan, females have equality in education, but yet in official

economy there is not fully partnership which is arise from conservative thoughts in relation with females and their labors.

Females only are holder of 1% of world property and only have share only in 10% of world income and only 14% of leadership positions in public of private sectors and this is in condition that females have produced half of food of world and only have 1% of land under their ownership.

Females not only in comparison with men have less time in achieving income, but when they work out of home, have the average income very lower than males.

Achievable observations indicate that there is sexual income gap in all countries and can be very different and even in some cases conversely. However, for example in Brazil, females under 25 years have more average hour wage to their male partners. Since females mostly are working in occupations with low wages, naturally have lower income to the males. Average per capita income by share of wages and non-agriculture incomes for Net national production is lower than mails.

According to UNICEF report (United National Children Organization) this calculation refers on basis of different in wage and partnership in work force that in Middle East evaluation of income resulted of female's work is about 30% of males. Problem of income gap between females and males is not allocated to Middle East region and or generally developing countries, but advanced countries such as US also are not excepted from this. However, annual average of income of full time females during a year in 2008 has been equal to 35.745 in comparison with males incomes in such situation has been 46.367 US. www.census.gov/prod/2009

In 2007, among 33.000876 employed couple, only in 35.6% of cases, female income has been more than their spouses. . (Bureau of Labor Statistics, 2009)

(Bureau of Labor Statistics, 2009)

In 2008, average weekly income for full time work of female has been 638 USD in comparison with 798 USD for males.

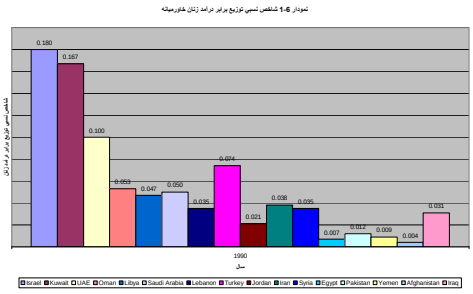
But what separates developed and developing countries in the field of income between male and female is the rate of this gap.

2. Economic Resources Dimension (Income)[2]

Fifth dimension of gender development of whole of index dimensions, is economic resource dimension which gains by share of male and female's income. This dimension measures approximate income achieved on the basis of ratio of female's income in non-agricultural division to male's in come in non-agricultural division, share of male and female in economic active population, total population of male and female and Internal Gross Production (IGP) on the basis of USD.

3. Investigation of Last Situation of Women in the Field of Income and Middle East

In diagram 1-6 relative index of equal distribution in 1990 which in this chapter we call it income index, is shown:



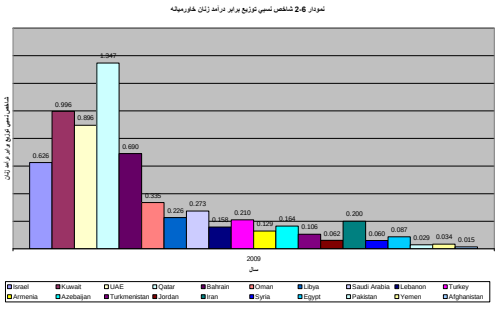
Higher figure of index belonging to Israeil, has been 0.180 and Kuwait and Arab Emirates in order is there in next ranks with female's income indices.

Female's income index of Iran in this year has been 0.038 and has 8th rank between 16 countries. Income indices of Afghanistan females also, with the amount of 0.004 having the least rate.

Income indices of woman of Qatar, Bahrain, Armenia, Palestine because of lack of needed data is not calculable.

4. Investigation of Present Situation of Female's Income in Middle East

In Figure 2-6 income index of Middle East Countries Female's income index in 2009 B.C. is shown:



The highest income index belongs to Qatar females in the rate of 1.347. Index figure above one indicates that female's income has been higher than males' and about Qatar also calculation of index indicates this. After Qatar, Kuwait and Arab Emirates by 0.996 and 0.896 indices there are in next rates.

Israel that in 1990 by females' income index of 0.180 has had the first rank, in 2009 by index of 0.626 has

gained the rank of 5 in region and indeed Israel index class in 1990-2009 has been declined.

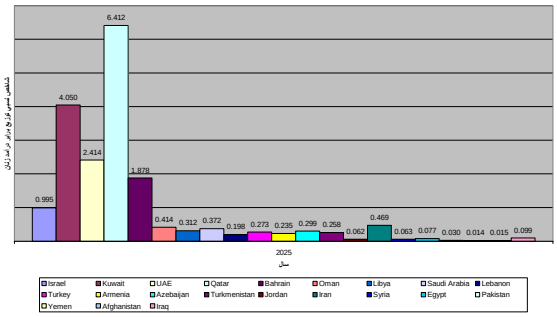
Iran's female income index also has reached to 0.200. The rank of Iran among 20 country of region has been 10 and in relative to 1990, has had decline. Possibility of calculating income index of Palestine and Iraq female also because of lack of related numbers there was not. The lowest income index also belongs to Afghanistan females with the amount of 0.015.

In table 1-6 income of female of 21 countries in Middle East in 1990-2009 and in 2009 is 0.332. Therefore, we can result that female's income of the region in above period and in comparison with men has been increased.

Also average of female's income in 1990 is the lowest and in 2009 has had the highest quantity. Average of income index of Israel females in 1990-2009, by quantity of 0.392 have the highest quantity and thus Kuwait and Qatar by quantities of 0.380 and 0.339 are in next ranks.

Income index of females of Iran during 1990-2009 has measured in the rate of 0.092. Iran among 21 countries has rank of 11. The result is that index of females of Iran about 1990-2009 and all years have been less than female's income index average.

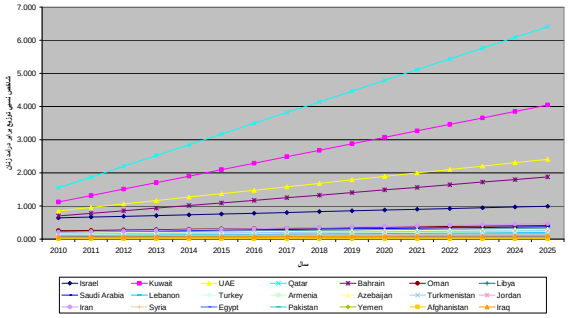
3-1-1-6 Forecasting of Future Situation of Female's Income of Middle East



In diagram 3-6 forecasting of income index of 21 countries of Middle East in 2025 has been shown.

The highest female's income index related to Qatar with unbelievable figure of 6.412 has been forecasted. After that countries of Kuwait and Arab Emirates are encountered with un-anticipated forecasting of 4.05 and 2.414. The lowest female's income index is forecasted in relation with Yemen and by figure 0.014.

Female of Iran Income Index in 2025 will reach to figure 0.469 and rank of Iran also is forecasted in region. For Palestine also there has not been possibility of forecasting female's income index Figure of 4-6 shows trend of income index trend of 21 countries of Middle East since 2010 up to 2025.



Countries index trend diagram is divided into two groups by trend of ascending and fix index. Index of Israel, Oman, Libya, Saudi Arabia, Kuwait, United Arab Emirates, Qatar, Bahrein, Lebanon, Turkey, Armenia, Azerbaijan, Turkmenistan, Iran, Saudi Arabia and index of Jordan, Syria, Egypt, Pakistan, Yemen and Afghnistan is forecasted fixed and without change.

2-1-6- Female Preventive Factors for Gaining Income Equal to Men

Many of performed researches is expressive the matter that in many economics specially in non-developed countries share of females partnership in performance of home works and without wage is more than males. Usually female are employing less than men in works that has regular wage and salary. Also, in economics based on agriculture which mainly is concentrates in tree continent of Asia, Africa and Latin America. Females even work more than men, but have less share of wages and incomes and in jobs similar to men also have less benefits and incomes and also in jobs similar to men have less benefits relative to men. Results of analysis of three indices of economic partnership, occupation and incomes is indicating of this reality that probability of women occupation in non-official economic is lack of legal and supervision frames, is more than males and the social security benefits is less and naturally their vulnerability is more. Therefore, females in many of societies form high ratio of low income labors and their income is not enough for themselves and their families.

Labor of female related to house works and without wages vastly in statistics is evident. Many efforts for documentation of these statistics on behalf of Development Fund for Females of United Nations has been performed which is including work without wage which refers to duties of females in home and house affairs, however mostly not as work but is known an activity.

- One of the preventive factors of females for gaining income equal to men is lack of presence of in official economy and in sections with high added value. In developed countries 6 women form each 10 women are occupied in non-official economy that really are

self employee. In private sector there are unfavorable conditions such as: low wage and possibility of little development for females. Tendency of females in work on sections with low added value is because of existence of legal barriers that make females without skill and achieving to necessary financial resources for business.

Many of these females are working in house works and non-official production centers, while other females are workers without wages in family economic enterprises and family fields.

In some non-agricultural sections usually females are more active than males. For example in Bangladesh among each 3 females two of them are working in cloth industry (World Bank, 2000). In south of Asia, North of Africa and Middle East only one female of 5 employed females are working in non-agricultural sections (United Nations, 2007).

Difference of wage between females and males and separation of jobs is main frame of female works in private sector. In Middle East, marriage of females and having children also is one the factors of non-employment of females. While in other regions of the world possibility of occupation of females after marriage or having children is became more.

- The other factors preventing females for having self-employee is expenses of this type of trade. For job making facilities between females a considerable effort for issuing legal and registered licenses is needed. Yet for females improvement in job and reaching to high degree of work and achieving to banking loan is difficult. (World Bank 2007 e:f)

Hdr-, 1995)

Most of low income females have been self employee and or are working in very little jobs and yet with regard to this point that more than 300 million low income females are active in very little business, only about 5,000,000 female have access to financial and monetary credit resources (mean of financial and monetary resources is are known banks and institutes and official and is not including usurers).

- Females also encountered with this fact that law and tradition in many cases in their access to product resources and mainly land are preventive and presenting services trade development services related to females is not enough. For promotion of females job making development of services related to development of females trade such as supporting business plans and labor and capability and access to facilities of business for females is needed.

- Females in sections with low added value are not able to reach to sections with higher added value. Singapore, Taiwan and Malaysia are good examples for this claim. Of course professional education is a necessary pre-condition for countries for passing of a job with low added value toward a job with high added value (World Bank, 2007:1620).

5. Gender Gap in Gaining Income

Most of people in the world in financial point of view are poor and human development indices follow removing of poverty in the world. Also females form main population of poor and achieving females to monetary and financial credits (specially, females with low income) is one of main factors in progress of human development. Statistics indicates that females in many cases can not benefit from income equal to females). In continents that most of their countries are considered under developing such as Asia, Africa and Sought of America, women form most part of work force but always there are in lower position than men in the case of income. In regions, such as Latin America and Karaeeb and South west of Asia, females form near 40% of work force in non-agricultural sections(United Nations, 2007:12).

In Arabic countries of Middle East also gender difference in rate of gained income is very ignorance and this is in a manner that Arabic contract of human rights in its article 32, among citizens for gaining income is not considered difference. So that in Article 32 of Arabic contract of human rights it is mentioned that governments should ensure their citizens in this case that none of them in occupation are not encountered with difference and all of them during joining of suitable wage in the case of equal works receive equal rights (Zakerian, Mehdi, 1382 p. 200).

Gender gap in most of societies prevent of presence of females in official sections and shift females toward non-official works and in other side females in non-official sections could not have equal income equal to their aligned men.

Gender gap even can indicate in rate of paid salary with regard to this fact that females more are working in non-official sections. (Kolen and Sirven, 2007; 16-17)

Differences in rate of paid salary, child birth leave and raise and unequal rules of retirement cause that female leave occupation. For example, employed females in cloth industry are employed in Bangladesh, because the low salary of females and their obedient cause their exploitation in these sections.

Employed people often in their employment have many non-security, and lack of enough recognition of their work mostly cause discriminate in relation with females for achieving labor market and work condition that knows females as a part of work force. Tendency of females in sections with low added value is because of legal barriers which make

females without skill and access to necessary financial resources for business. However many social and cultural factors in private sector also interferes on type of females employing.

For example according to documents in countries where their females relatively marry later, percent of female labors of non-agricultural section is higher (OECD, 2006).

In addition in recent studies of Economic and Development Cooperation Organization it is has been mentioned that single females more are employed in official economic while females having more children are working in non-official economy (OECD, 20008b).

Of course some studies also indicates that income gap between males and females is not only because of gender discrimination but is house working and care of children responsibilities of females have been more than males and restricts female in choosing type of work they perform. (Institute for Females's Policy Research 2006)

In deed dimensions such as occupation and economic partnership and making decision and rate of gained income of females as chain are joined to each other and creating disorder in one of these dimensions can cause failure of other dimensions.

Therefore it is needed that for each of barriers factor we seek a remedy.

- By basic changes in legal frames facilities we can increase share of women in sections with high add value inside of official section such as non-official section and with respect to long term and middle term strategies it is required that more research about structural reasons of this subject be accomplished that why most of females are employed in non-official sections.

- Governments together with educational organizations, education institutes and trade banks try in direction of increasing of females' partnership in non-agricultural sections.

- Organizations that support females economic job makers in official and non-official sections, themselves be supported.

- Decreasing in expenses related to business for helping entrance of females to work area by removing business registration barriers and related licenses and also gaining ownership of land, etc.

- To ensure of observance of these in future policies some countries are adjusting program by special emphasize upon gender aspects.

Recently, Lesoto has facilitated registration of land for females and allowed that married females can transfer title deed without signature of their spouse. In addition many of advanced economics such as Austria, Canada and or Newzeland have introduced simple actions for registration of enterprise and business and have approved that may have important and negative effect on employment of females. However this has been approved in Antep in Uganda. This project by awarding international capital has became successful. This flowing wave is performed for females for the first time. (World Bank, 2006:42)

4-1-6- Relation of Education and Income

As it was referred, triple dimension of female's occupation, economic partnership and female's making decision and gained incomes by females join to each other like a chain. But what is starter of these types of activities, is dimension of education, because literacy of females helps them achieve to each of other dimension brought up in gender development of total index.

- Training cause that utilization of females in each job such as work in relation with market and other jobs (private job, family, etc…) be increased.

Also training and education of female causes that achieving female to wage labors became more and their receipts also increase.

- Education is one of the effective factors in rate of received income of female and statistics indicates that whatever the education of them is more, they would benefit from more income. (Bureau of Labor Statistics, 2009).

One year of more education of female in Pakistan has shown that their salary has increased 20%. Also a similar investigation in developing countries Sahel Aj, India, Indonesia and Thiland shows that one year of females' education causes approximately 15% increase in income while in comparison with male would be 11% (Hdr-1995).

Change of force work structure and entrance of female to various fields of work and effort, necessity of female's literacy, higher and professional education make them necessary. Higher education also cause increase of their economic partnership in management and professional level and women by more economic partnerships can have higher income and be effective on promotion of family. Female also can have familiarization with their rights in society and adjudicate their rights and in this way can send among themselves represents to parliaments to more than before can survive the rights of females and children.

In deed change of work force structure will follow social structure change, because cause change of attitude of policy makers and programmer to the challenges on the way of females and their success in achieving to educational, occupation, economic and political partnerships opportunities will be effective on creating human security and prestige of females,

observance of their rights is labor market, right of females in family and society.

Increasing of female's capabilities, male head societies that only were utilized of half of their human capital, used these human wealth and circulated better society economic wheels faster and better. Presence of female in social and economic and also political fields helps them to be better mothers for educating and health of their children and educate future generation a literate and skillful and trained generation.

Investigations shows that without improvement in the field of occupational opportunities for females for assist them in achieving useful and wage generating jobs in equal conditions with men, joining of human security and prestige and a respectful life and decreasing of poverty would not be ascertained up to 2015.

Policy makers can by creating job opportunities for males and wage rate equal to females endeavor in providing economic for females.

21th century is century of economic competitions and countries would have strength of economic competitions that can have more advanced human. This advancement in human quality requires recognition of advancing indices and as many of advanced countries specially in Northern Europe showed the first phase have been human development and then gender development in which capabilities of females has appeared and each unit of population could prove its applicable role by his/her qualification.

Islamic Republic of Iran also by attention to barriers of females development in gaining income equal to men has opened the way for economic partnership and making decision for females to female join rights equal to men. Assurance of achievement to these rights is presence of females in Parliament to both achieve to another one of gender development indices and that by their presence in legislation parliament can bring up better and newer laws for equality of females and men.

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Mathematical analysis of Solutions of Drug Models

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Abstract: In this paper the behavior of solutions of permanent drug resistance model is discussed.The equilibrium points of two drugs resistance are computed. The local stability near equilibrium pionts is discussed. The boundedness, existance of periodic orbits, global stability of permanent drug resistance are studied. the probability generating function for two drugs resistance model in all possible cases is discussed. The obtained results improve and generalize some known results in the literature.
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1 . Introduction

The emergence of drug resistance has created a new challenge for experimental and theoretical studies. A great important in therapy have been in tumors of hemopoietic and lymphoreticular systems in a number of childhood and in germ cells tumors ([1]). However there has been relative little success in the case of clinical detectable dimension solid tumors. One of the reasons which can lead to the failure of chemotherapy is the possible resistance of the tumor cells to the effect of the drug. In [2], the authors used a scheme proposed for self-renewing system in which cells be either (i) system cells, (ii) early differentiated cells, or (iii) end cells, and the human tumor cells model more randomly between these three compartments with transitions in one way (i)→(ii)→(iii) (see [3] and [4]). Following [5], we assume that each tumor arises from a single cell. This may say that the first tumor cell is a stem cell and all the other cells derive from this single stem cell. More precisely, it is assumed that the divisions occur at a rate b and the rate of transmission to nonstem cells is denoted by d. So the system growth can be seen as birth process with parameter b and death one with parameter d. By a resistant cell we mean a cell which will survive administration of the drug at a therapeutic dose with propability one. Sometimes in studying the control of the emergence of drug resistence pathogen, it is
important to understand the nonlinear transmission dynamics of both the drug-sensitive and the drug-resistant pathogen. We will consider the case for which both resistant and sensitive stem cells divide and grow at the same rate. We also assume that the coversion to resistance occurs spontaneously during the intermitotic period. Moreover unlike some of the previous models , we consider the availability of possible interdivisional mechanism [6].

In this paper we consider the case where a single drug is available. Define a as the rate of development of spontaneous resistance for stem cells to a given drug, which may also be referred as the mutation rate to drug resistance. Let $S(t)$ be the deterministic size of the stem cell compartment at time t. Consider two drugs T_1 and T_2, say; then four resistant exist: (i) stem cells sensitive to both drugs, S; (ii) stem cells resistant to the first but not the second drug, R_1; (iii) stem cells resistant to the second but sensitive to the first R_2; (iv) cells resistant to both drugs, R_{12}. Analogously to the single drug situation, define transition rates α_1 and α_2 for sensitive cells to become resistant to T_1 and T_2 respectively. Let α_1 and α_2 be the rates at with cells resistant to T_1 and T_2, become resistant to other agent. We will assume that cells may not develop resistance to both agents simultaneously. We will also assume that all resistant cells grow at the same rate as the sensitive cells. Define

$$P_{ijk}(t) = P\{R_1(t) = i, R_2(t) = j, R_{12}(t) = k\},$$

and

$$\phi(t, s_1, s_2, s_3) = \sum_{i=0}^{\infty}\sum_{j=0}^{\infty}\sum_{k=0}^{\infty} P_{ijk}(t)s_1^i s_2^j s_3^k \quad (1.1)$$

Then using the Kolmogorov backward equations, we have

$$\frac{dP_{ijk}(t)}{dt} = (\alpha_1 + \alpha_1)S(t)P_{ijk}(t) - (b + d + a_1)iP_{ijk}(t)$$
$$-(b + d + \alpha_2)jP_{ijk}(t) - (b + d)kP_{ijk}(t)$$
$$+\alpha_1 S(t)P_{i-1jk}(t) + \alpha_2 S(t)P_{ij-1k}(t)$$

$$+b(i-1)P_{i-1jk}(t)+b(j-1)P_{ij-1k}(t)+ b(k-1)P_{ijk-1}(t)$$

$$+d(i+1)P_{i+1jk}(t)+d(j+1)P_{ij+1k}(t)+ d(k+1)P_{ijk+1}(t) \qquad (1.2)$$

$$+a_1(i+1)P_{i-1j+1k-1}(t)+a_2(j+1)P_{ij+1k-1}(t).$$

As before, set $P_{ijk}(t)=0$ for $i<0, j<0$ or $k<0$. Multiplying both sides by $s_1^i s_2^j s_3^k$ and summing $\sum_{i=0}^{\infty}\sum_{j=0}^{\infty}\sum_{k=0}^{\infty}$ yields

$$\frac{\partial\phi}{\partial t}+[(1-s_1)(bs_1-d)+a_1(s_1-s_2)]\frac{\partial\phi}{\partial s_1}+[(1-s_2)(bs_2-d)+a_2(s_2-s_3)]\frac{\partial\phi}{\partial s_2}+(1-s_3)(bs_3-d)\frac{\partial\phi}{\partial s_3} \qquad (1.3)$$

$$=S(t)[a_1(s_1-1)+a_2(s_2-1)].$$

Again we will use Cauchy's method of characteristics to solve this equation. Let $x_1=t$, $x_2=s_1$, $x_3=s_2$ and $x_4=s_3$ and we have the differential system of two drugs in the form

$$\begin{aligned}
\frac{dx_1}{dt}&=1\\
\frac{dx_2}{dt}&=(1-x_2)(bx_2-d)+a_1(x_2-x_3),\\
\frac{dx_3}{dt}&=(1-x_3)(bx_3-d)+a_2(x_3-x_4)\\
\frac{dx_4}{dt}&=(1-x_4)(bx_4-d),\\
\frac{d\phi}{dt}&=S(x_1)[a_1(x_2-1)+a_2(x_3-1)],
\end{aligned} \qquad (1.4)$$

where the growth of the stem cell compartment may be viewed as a birth and death process with rates b and d respectively. Without loss of generality we set $x=x_2$, $y=x_3$ and $z=x_4$ we have the reduced system

$$\begin{aligned}
\frac{dx}{dt}&=(1-x)(bx-d)+a_1(x-z),\\
\frac{dy}{dt}&=(1-y)(by-d)+a_2(y-z),\\
\frac{dz}{dt}&=(1-z)(bz-d).
\end{aligned} \qquad (1.5)$$

The organization of the paper is as follows. In the next section, we discuss the existence of equilibria and their local and global stability . In the section that follows, we calculate the probability generating function for two drugs resistance model in all possible cases of equilibria. Then we give a conclusion for our results.

2. **The equilibria: existence and Stability**

The equilibria of system (1.5) are obtained by solving the system of isocline equations

$$\begin{aligned}
(1-x)(bx-d)+a_1(x-z)&=0,\\
(1-y)(by-d)+a_2(y-z)&=0,\\
(1-z)(bz-d)&=0.
\end{aligned} \qquad (2.1)$$

The possible equilibria are of the form $E_1=(1,1,1)$, $E_2=\left(\frac{a_1+d}{b},1,1\right)$, $E_3=\left(1,\frac{a_2+d}{b},1\right)$, $E_4=\left(\frac{a_1+d}{b},\frac{a_2+d}{b},1\right)$, $E_5=\left(\frac{d}{b},\frac{d}{b},\frac{d}{b}\right)$, $E_6=\left(\frac{d}{b},\frac{a_2+b}{b},\frac{d}{b}\right)$, $E_7=\left(\frac{a_1+b}{b},\frac{d}{b},\frac{d}{b}\right)$ and $E_8=\left(\frac{a_1+b}{b},\frac{a_2+b}{b},\frac{d}{b}\right)$. The existence and local stability of the prospective equilibria are analysed as follows. The Jacobian matrix due to the linearization of (1.5) about an arbitrary equilibrium $E=(x,y,z)\in R_+^3$ is given by

$$J_{E=(x,y,z)}=\begin{pmatrix} b(1-x)-(bx-d)+a_1 & 0 & -a_1\\ 0 & b(1-y)-(by-d)+a_2 & -a_2\\ 0 & 0 & b(1-z)-(bz-d)\end{pmatrix}. \qquad (2.2)$$

Let J_i denotes $J_{E=(x,y,z)}$ at E_i, $i=1,2,3,4,5,6,7,8$ respectively. Assuming that the difference between the birth and death rates is $\delta=b-d\geq 0$,then from (2.2) we have

$$J_1=\begin{pmatrix} a_1-\delta & 0 & -a_1\\ 0 & a_2-\delta & -a_2\\ 0 & 0 & \delta\end{pmatrix},$$

$$J_2=\begin{pmatrix} -\delta-a_1 & 0 & -a_1\\ 0 & a_2-\delta & -a_2\\ 0 & 0 & \delta\end{pmatrix},$$

$$J_3=\begin{pmatrix} a_1-\delta & 0 & -a_1\\ 0 & \delta & a_2\\ 0 & 0 & -\delta\end{pmatrix},$$

$$J_4=\begin{pmatrix} \delta-a_1 & 0 & -a_1\\ 0 & \delta & a_2\\ 0 & 0 & -\delta\end{pmatrix},$$

$$J_5=\begin{pmatrix} \delta+a_1 & 0 & a_1\\ 0 & \delta+a_2 & -a_2\\ 0 & 0 & \delta\end{pmatrix},$$

$$J_6=\begin{pmatrix} \delta+a_1 & 0 & -a_1\\ 0 & -\delta-a_2 & -a_2\\ 0 & 0 & \delta\end{pmatrix},$$

$$J_7=\begin{pmatrix} -\delta-a_1 & 0 & -a_1\\ 0 & \delta+a_2 & -a_2\\ 0 & 0 & \delta\end{pmatrix},$$

$$J_8=\begin{pmatrix} -\delta-a_1 & 0 & -a_1\\ 0 & \delta & a_2\\ 0 & 0 & \delta\end{pmatrix}.$$

Form the above matrices, we have:

(1) The eigenvalues at $E_1=(1,1,1)$ are $\lambda_1=-\delta,\ \lambda_2=a_1-\delta$ and $\lambda_3=a_2-\delta$.

(2) The eigenvalues at $E_2=\left(\frac{a_1+d}{b},1,1\right)$ are $\lambda_1=-\delta,\ \lambda_2=-\delta+a_1$ and $\lambda_3=\delta-a_2$.

(3) The eigenvalues at $E_3=\left(1,\frac{a_2+d}{b},1\right)$ are $\lambda_1=-\delta,\lambda_2=\delta-a_1$ and $\lambda_3=\delta-a_2$.

(4) The eigenvalues at $E_4 = \left(\frac{a_1+d}{b}, \frac{a_2+d}{b}, 1\right)$ are
$\lambda_1 = -\delta,\ \lambda_2 = \delta - a_1$ and $\lambda_3 = \delta - a_2$.
(5) The eigenvalues at $E_5 = \left(\frac{d}{b}, \frac{d}{b}, \frac{d}{b}\right)$ are
$\lambda_1 = \delta,\ \lambda_2 = \delta + a_1$ and $\lambda_3 = \delta + a_2$.
(6) The eigenvalues at $E_6 = \left(\frac{d}{b}, \frac{a_2+b}{b}, \frac{d}{b}\right)$ are
$\lambda_1 = \delta, \lambda_2 = \delta + a_1$ and $\lambda_3 = -\delta - a_2$.
(7) The eigenvalues at $E_7 = \left(\frac{a_1+b}{b}, \frac{d}{b}, \frac{d}{b}\right)$ are
$\lambda_1 = \delta,\ \lambda_2 = \delta + a_2$ and $\lambda_3 = -\delta - a_1$.
(8) The eigenvalues at $E_8 = \left(\frac{a_1+b}{b}, \frac{a_2+b}{b}, \frac{d}{b}\right)$ are
$\lambda_1 = \delta,\ \lambda_2 = -\delta - a_1$ and $\lambda_3 = -\delta - a_2$.
From the above discussion we have the following conclusion.

Proposition 2.1 Whenever $\delta \to 0$, then $E_5 \to E_1$ and so the system (1.5) have seven equilibria. Moreover if $\delta \to 0$ and $a_1 = a_2$, then $E_4 = E_8$ and the system has only six equilibria. Whenever $\delta > 0$ the system (1.5) have eight equilibria, where

for $\delta=\max(a_1,a_2)$, E_1 is locally asymptotically

stable while for $\delta \in (a_1, a_2)$ E_3 is locally asymptotically stable. For $\delta \in (a_1, a_2)$, E_2 is locally asymptotically stable. For $\delta < a_i$, $i = 1,2$ E_4 is locally asymptotically stable. The equilibria E_i, $i = 5,6,7,8$ are unstable. Now we discuss the boundedness of solutions of (1.5).

Theorem 2.1 All solution of (1.5) which initiate in R^3 are uniformly bounded.
Proof. Define a function
$w = x + y + x$ (2.3) (2.3)
The time derivative of (2.3) along the solutions of (1.5) is
$\frac{dw}{dt} = (b+d)w - b(x^2 + y^2 + z^2) + a_1(x-2) + a_2(y-2) - 3d$

$\leq (b+d)w + a_1 x + a_2 y - (a_1 + a_2)z.$
For each $D > 0$, the following inequality holds
$\frac{dw}{dt} + (D - (b+d))w \leq (a_1 + D)x + (a_2 + D)y - (a_1 + a_2 - D)z$
(2.4) (2.4)
Now if we take $\max(b+d) < D < min(a_1 + a_2)$, the Eq. (2.4) reduced to
$$\frac{dw}{dt} + \tilde{D}w \leq (a_1 + D),$$
where $\tilde{D} = D - (b+d)$. Then we can find a constant $L > 0$, say such that $\frac{dw}{dt} + \tilde{D}w$. Applying the theorem of differential inequality (see[7] and [8]) we obtain
$0 < w(x,y,z) \leq \frac{L}{\tilde{D}}\left(1 - e^{-\tilde{D}t}\right) + w(x(0), y(0), z(0))e^{-\tilde{D}t},$

and for $t \to \infty$, we have
$0 < w < \frac{L}{\tilde{D}}.$
Hence all solutions of (1.5) that initiate in $\{R_+^3 - 0\}$ are confined in the region.

Now we use the idea of [12] and write the system (1.5) in the form
$\frac{dz}{dt} = (1-u)(bu - d) = p(x_i, z),$ (2.5)
$\frac{dx_i}{dt} = (1 - x_i)(bx_i - d) + a_1(x_i - z) = p(x_i, z)$
$i = 1,2.$ and have the following result regarding the nonexistence of periodic orbits.

Theorem 2.2 The system (2.5) does not have nontrivial periodic orbits.
Proof. Consider the system (2.5) for $x_i > 0$ and $z > 0$. Taking a Dulac function
$$D(x_i, z) = e^{\frac{2b}{a_1}x_i z}.$$
Then
$div[D(x,y)F(x,y)]$

$$= div \frac{D(x,y)[(1-x)(bx-d) + a_1(x_i - z)]}{D(x,y)[(1-z)(bz-d)]}$$
$$= \frac{\partial(D_p)}{\partial x} + \frac{\partial(D_q)}{\partial x}$$
$$= -be^{\frac{2b}{a_1}xz}[x^2 - Ax - B].$$
where $A = 1 + \frac{2(b-d)}{a_1} > 0$ and $B = 2 + \frac{3d+2a_1}{b} > 0$. Therefore for the value of x in the interval $\left(\frac{A}{2} - \frac{1}{2}\sqrt{A^2 + 4AB}, \frac{A}{2} + \frac{1}{2}\sqrt{A^2 + 4AB}\right)$ we have
$$\frac{\partial(D_p)}{\partial x} + \frac{\partial(D_q)}{\partial x} < 0,$$
Thus by Bendixon-Dulac Theorem ([8]) the conclusion follows.

Now we have seen the local stability of all the equilibria of the 3-dimensional system (1.5), but it is interesting to know about the global stability of these equilibria. Our approach depends on the Lozinski measure ([8]).
Applying this measure on the variational matrix J_1 we obtain
$\mu_1(A) = \max\{a_1 - \delta, a_2 - \delta, a_1 + a_2 - \delta\}.$
Since $a_1 > 0$, $a_2 > 0$ and $\delta > 0$, then clearly $\mu_1(A) = a_1 + a_2 - \delta$ and if $a_1 + a_2 < \delta$, then $E_1 = (1,1,1)$ is globally asymptotically stable. This with the discussion in Proposition 2.1 shows that if $\delta > a_1 + a_2$, then $E_1 = (1,1,1)$ is locally and globally asymptotically stable.

Thus we can summurize the situation about the eight equilibria when δ, the difference between the rate of birth and death rate is positive, as follows

(1)

n the case of $\delta > A$ where $A = \max\{a_1, a_2\}$, $E_1 = (1,1,1)$ is locally asymptotically stable. Moreover if δ going to be larger such that $\delta > a_1 + a_2$, then by Lozinski measure, $E_1 = (1,1,1)$ is globally asymptotically stable.

(2)

f $a_1 > \delta > a_2$, then $E_2 = \left(\frac{a_1+d}{b}, 1, 1\right)$ is locally asymptotically stable.

(3)

f $a_2 > \delta > a_1$, then $E_3 = \left(1, \frac{a_2+d}{b}, 1\right)$ is locally asymptotically stable, while if $\delta > a_1 + a_2$, then $E_3 = \left(1, \frac{a_2+d}{b}, 1\right)$ is globally asymptotically stable.

(4)

f $\delta < a_i, i = 1,2$, then $E_4 = \left(\frac{a_1+d}{b}, \frac{a_2+d}{b}, 1\right)$ is locally asymptotically stable.

(5)

he remaining equilibrium points E_5, E_6, E_7 and E_8 are unstable.

3. Probability generating function at equilibria

In this section we calculate the probability generating function $\phi(t,x,y,z)$ for the two drugs resistance model in all possible cases. Following Coldman et al [5] we consider the probability generating function in the form

$$\phi(t,x,y,z) = \exp\{I_1(t) + \alpha_1 I_2(a_1,x) + \alpha_2 I_2(a_2,y)\}, \quad (3.1)$$

where

$$I_1(t) = -\delta(\alpha_1+\alpha_2)(z-1)\int_0^t \frac{S(t-u)}{I_3(u)}du, \quad (3.2)$$

$$I_2(a,s) = \int_0^t \frac{S(t-u)e^{(\delta-a)u}[I_3(u)]^{-2}}{\left([\delta^2(s-z)]^{-1} - b\int_0^u e^{(\delta-a)v}[I_3(v)]^{-2}dv\right)}du, \quad (3.3)$$

$$I_3(u) = b(z-1)e^{\delta u} - (bz-d), \quad (3.4)$$

(3.4)

$\delta = b - d$, $\alpha = (\alpha_1$ or $\alpha_2)$, $s = (x$ or $y)$ and $S(t) = Ae^{\delta t}$ with $A = S(0)$. The probability that there are no resistant cell present at time t, $\{\phi(t,0,0,1)\}$ is an upper bound to the probability that the tumor will eliminated by the drug under consideration. Therefore the value $\phi(t,0,0,1)$ is of considerable interest during the following discussion.

Now, we consider the following cases:

Case 1. If $d \neq 0$, $b = 0$, $\alpha_1 = 0$ and $\alpha_2 = 0$, then $\delta = -d$ and the system (1.5) becomes

$$\frac{dx}{dt} = -d(1-x)$$
$$\frac{dy}{dt} = -d(1-y)$$
(3.5)
$$\frac{dz}{dt} = -d(1-z).$$

Let us consider the initial conditions

$x(0) = \beta_1, y(0) = \beta_2, z(0) = \beta_3$.

Then the solutions of (3.5) are

$x(t) = 1 + (\beta_1 - 1)e^{dt}$,
$y(t) = 1 + (\beta_2 - 1)e^{dt}$ and
$z(t) = 1 + (\beta_3 - 1)e^{dt}$.

Now, form (3.2), (3.3) and (3.4) we obtain

$I_3(u) = d$,
$I_1(t) = A(\beta_3 - 1)t$,
$I_2(a_2,x) = \frac{A}{d}(\beta_1 - \beta_3)(1 - e^{-dt})$,
$I_2(a_2,y) = \frac{A}{d}(\beta_2 - \beta_3)(1 - e^{-dt})$,

and

$$\phi(t,x,y,z) = \exp\left\{A(\beta_3-1)t + (\alpha_1+\alpha_2)t + \alpha_1\frac{A}{d}(\beta_1-\beta_3)(1-e^{-dt}) + \alpha_2\frac{A}{d}(\beta_2-\beta_3)(1-e^{-dt})\right\} \quad (3.6)$$

Remark 3.1 From the above probability generating function we have

(i) If $x = y = z = 1 + (k-1)e^{dt}$, then (3.6) takes the form $\phi(t,x,x,x) = \exp[A(k-1)(\alpha_1+\alpha_2)t]$,

(ii) $\phi(0,x,y,z) = 1$.

Case 2. If $d = 0$, $b \neq 0$, $\alpha_1 = 0$ and $\alpha_2 = 0$, then $\delta = d$ and the system (1.5) becomes

$$\frac{dx}{dt} = -bx(1-x)$$
$$\frac{dy}{dt} = -by(1-y) \quad (3.7)$$
$$\frac{dz}{dt} = -bz(1-z).$$

Then the solutions of (3.7) are

$x(t) = \beta_1 e^{bt}\left(1 + \beta_1(e^{bt}-1)\right)^{-1}$,
$y(t) = \beta_2 e^{bt}\left(1 + \beta_2(e^{bt}-1)\right)^{-1}$,
$z(t) = \beta_3 e^{bt}\left(1 + \beta_3(e^{bt}-1)\right)^{-1}$.

Now, form (3.2), (3.3) and (3.4) we obtain

$$I_3(u) = \frac{-be^{bu}}{1+\beta_3(e^{bu}-1)}$$

$$I_1(t) = -\frac{A(\alpha_1-\alpha_2)(\beta_3-1)}{1+\beta_3(e^{bu}-1)}\left(\frac{\beta_3-1}{b}(e^{bt}-1) - \beta_3 t e^{bt}\right)$$

$$I_2(0,s) = Ae^{bt}\int_0^t \frac{e^{-au}\left(1+\beta_3(e^{bt}-1)\right)^2}{\frac{e^{2bu}}{s-z} - be^{2bu}\int_0^u e^{(b-a)v}\left(1+\beta_3(e^{bv}-1)\right)^2 dv}du,$$

and

$\phi(t,x,y,z) = \exp\{I_1(t) + \alpha_1 I_2(0,x) + \alpha_2 I_2(0,y)\}$.
(3.8)

Remark 3.2 From the above probability generating function we have
(i) If $x = y = z = 1 + (k-1)e^{dt}$, then (3.8) takes the form $\phi(t,x,x,x) = \exp\{A(k-1)(\alpha_1+\alpha_2)t\}$,
(ii) $\phi(0,x,y,z) = 1$.
(iii) $\phi(t,1,1,1) = 1$.

Case 3. If $d \neq 0$, $b \neq 0$, $\alpha_1 = 0$ and $\alpha_2 = 0$, then $\delta = b - d$ and the system (1.5) becomes

$$\frac{dx}{dt} = (1-x)(bx-d),$$
$$\frac{dy}{dt} = (1-y)(by-d), \qquad (3.9)$$
$$\frac{dz}{dt} = (1-z)(bz-d).$$

Then the solutions of (3.9) are

$$x(t) = 1 + \frac{(d-b)(\beta_1-1)}{b(\beta_1-1)+(d-b\beta_1)e^{(b-d)t}},$$
$$y(t) = 1 + \frac{(d-b)(\beta_2-1)}{b(\beta_2-1)+(d-b\beta_2)e^{(b-d)t}},$$
$$z(t) = 1 + \frac{(d-b)(\beta_3-1)}{b(\beta_3-1)+(d-b\beta_3)e^{(b-d)t}}.$$

Now, form (3.2), (3.3) and (3.4) we obtain

$$I_3(u) = \frac{(d-b)^2 e^{(b-d)u}}{b(\beta_3-1)+(d-b\beta_3)e^{(b-d)u}},$$
$$I_1(t) = \frac{(\alpha_1+\alpha_1)}{(d-b)^2}(\beta_3-1)I_3(t)\left[(d-b\beta_3)t + b(\beta_3-1)e^{(b-d)t} - 1\right],$$

$$I_2(0,s) = Ae^{(b-d)t}\int_0^t \frac{[I_3(u)]^{-2}}{[(b-d)^2(s-z)]^{-1} - b\int_0^u e^{(b-d)v}[I_3(v)]^{-2}dv}du,$$

and
$\phi(t,x,y,z) = \exp\{I_1(t) + \alpha_1 I_2(0,x) + \alpha_2 I_2(0,y)\}$.
(3.10)

Remark 3.3 From (3.10) we get the following
(i) $\phi(0,x,y,z) = 1$.
(ii) If $\beta_3 = \frac{d}{b}$, then $z = \frac{d}{b}$ and $\phi\left(t,x,y,\frac{d}{b}\right) = \exp\left\{\frac{A}{d-b} + (\alpha_1(1-\beta_1) + \alpha_2(1-\beta_2)\left(e^{(b-d)t}-1\right)\right\}$

(iii) From the above relation, we get
$\phi(t,1,1,\frac{d}{b}) = 1$.

Case 4. If $d \neq 0$, $b = 0$, $\alpha_1 \neq 0$ and $\alpha_2 = 0$, then $\delta = -d$ and the system (1.5) becomes

$$\frac{dx}{dt} = -d(1-x) + \alpha_1(x-y),$$
$$\frac{dy}{dt} = -d(1-y), \qquad (3.11)$$
$$\frac{dz}{dt} = -d(1-z).$$

Then the solutions of (3.11) are

$$x(t) = 1 + (\beta_3 - 1)e^{dt} + (\beta_1 - \beta_3)e^{(d+\alpha_1)t},$$
$$y(t) = 1 + (\beta_2 - 1)e^{dt},$$
$$z(t) = 1 + (\beta_3 - 1)e^{dt}.$$

Now, form (3.2), (3.3) and (3.4) we obtain

$$I_3(u) = d,$$
$$I_1(t) = \frac{A}{\alpha_1}(\alpha_1+\alpha_1)(\beta_2-1)(1-e^{-\alpha_1 t}),$$
$$I_2(\alpha_1,x) = \frac{A(\beta_1-\beta_2)}{d+\alpha_1}\left(1 - e^{-(d+\alpha_1)t}\right),$$
$$I_2(0,y) = \frac{A}{d}(\beta_2-\beta_3)(1-e^{-dt}),$$

and

$$\phi(t,x,y,z) = \exp\left\{\begin{matrix}\frac{A}{\alpha_1}(\alpha_1+\alpha_1)(\beta_2-1)(1-e^{-\alpha_1 t}) \\ +\frac{A(\beta_1-\beta_2)}{d+\alpha_1}\alpha_1\left(1-e^{-(d+\alpha_1)t}\right) \\ +\frac{A}{d}(\beta_2-\beta_3)\alpha_2(1-e^{-dt})\end{matrix}\right\}. \qquad (3.9)$$
(3.12)

Remark 3.4 It follows from (3.12) that
(i) $\phi(t,1,1,1) = 1$ for $\beta_1 = \beta_2 = \beta_3 = 1$.
(ii) $\phi(0,x,y,z) = 1$.
(iii) If $y = 0$ at $t = t_1$, then

$$\phi(t_1,x,0,z) = \exp\left\{\begin{matrix}\frac{A}{\alpha_1}(\alpha_1+\alpha_1)(\beta_3-1)(1-e^{-\alpha_1 t_1}) \\ +\frac{A(\beta_1-\beta_2)}{d+\alpha_1}\alpha_1\left(1-e^{-(d+\alpha_1)t_1}\right) \\ +\frac{A}{d}(\beta_2-\beta_3)\alpha_2\beta_2\end{matrix}\right\}.$$

Now, we use the above cases to compute the probability generating functions at the equilibrium points which help in calculating the probability that resistance is generated after treatment.

(i) The probability generating function at $E_1(1,1,1)$:

Using (3.1), (3.2), (3.3) and (3.4), if the probability generating function at $E_1(1,1,1)$ is $\phi_1(t,1,1,1)$, then

$$\phi_1(t,1,1,1) = \exp\{I_1(t) + \alpha_1 I_2(\alpha_1,x) + \alpha_2 I_2(\alpha_2,y)\},$$

where
$I_1(t) = 0$,
$I_2(\alpha_1,x) = I_2(\alpha_1,1) = 0$,
$I_2(\alpha_2,y) = I_2(\alpha_2,1) = 0$.
Hence from the definition of probability generating function , we get

$$\phi_1(t,1,1,1) = 1.$$

Similarly (3.11)

(ii) The probability generating function at

$E_2(\frac{a_1+d}{b},1,1)$is
$\phi_2\left(t,\frac{a_1+d}{b},1,1\right)=exp\{I_1(t)+\alpha_1 I_2(a_1,x_1)+\alpha_2 I_2(a_2,x_2)\}$,

such that
$I_1(t)=0, I_2(a_2,y)=I_2(a_2,1)=0,$
$I_2(a_1,x)-I_2(a_1,\frac{a_1+d}{b})-\frac{(a_1+d-x)}{b}\int_0^t S(t-u)du.$
Then
$\phi_2\left(t,\frac{a_1+d}{b},1,1\right)=\exp\{\alpha_1\frac{(a_1+d-b)}{b}\int_0^t S(t-u)du\}.$

Now, if we consider the case $S(t)=Ae^{(\delta-a_1)t}$, then $I_2(a_1,\frac{a_1+d}{b})=\frac{A}{b}[1-e^{(\delta-a_1)t}]$.
Hence again from the definition of probability generating function , we obtain
$\phi_2\left(t,\frac{a_1+d}{b},1,1\right)=\exp\{-\alpha_1\frac{A}{b}[e^{(\delta-a_1)t}-1]\}.$
Thus at equilibrium point $E_2(\frac{a_1+d}{b},1,1)$, if $a_1=\delta$, then the probability that single resistance will persist in is independent on δ .

(iii) The probability generating function at $E_3(1,\frac{a_2+d}{b},1)$is
$\phi_3(t,1,\frac{a_2+b}{b},1)=\exp\{I_1(t)+\alpha_1 I_2(a_1,x)+\alpha_2 I_2(a_2,y)\}$,

where $I_1(t)=0,\quad I_2(a_1,x)=I_2(a_1,1)=0$ and $I_2(a_2,y)=I_2(a_2,\frac{a_2+d}{b})=\frac{A}{b}[1-e^{(\delta-a_2)t}]$. Hence as above , we get
$\phi_3(t,1,\frac{a_2+d}{b},1)=\exp\{-\alpha_2\frac{A}{b}[e^{(\delta-a_2)t}-1]\}.$
Thus at equilibrium point $E_3(1,\frac{a_2+d}{b},1)$, if $a_2=\delta$, then the probability that single resistance will persist in is independent on δ.

(iv) The probability generating function at $E_4(\frac{a_1+d}{b},\frac{a_2+d}{b},1)$:
$\phi_4(t,\frac{a_1+d}{b},\frac{a_2+d}{b},1)=\exp\{I_1(t)+\alpha_1 I_2(a_1,x)+\alpha_2 I_2(a_2,y)\}$,

where $I_1(t)=0$. Under the conditions $\alpha_1=\delta$ and $\alpha_2=\delta$ as in the case of E_2 and E_3 , we get
$I_2(a_1,x)=\frac{A}{b}[1-e^{(\delta-a_1)t}],$
$I_2(a_2,y)=\frac{A}{b}[1-e^{(\delta-a_2)t}].$
Hence
$\phi_4(t,\frac{a_1+d}{b},\frac{a_2+d}{b},1)=\exp\{\frac{a_1A}{b}[e^{(\delta-a_1)t}-1]-\frac{a_2A}{b}[e^{(\delta-a_2)t}-1]\}.$

Thus at equilibrium point $E_4(\frac{a_1+d}{b},\frac{a_2+d}{b},1)$, if $a_1=a_2=\delta$, then the probability that single resistance will persist in is independent on δ .

(v) The probability generating function at $E_5(\frac{d}{b},\frac{d}{b},\frac{d}{b})$:
$\phi_5(t,\frac{d}{b},\frac{d}{b},\frac{d}{b})=\exp\{I_1(t)+\alpha_1 I_2(a_1,x)+\alpha_2 I_2(a_2,y)\}$,

where $I_1(t)=\frac{-\delta(a_1+a_2)}{b}\int_0^t S(t-u)du$ and $I_2(a_1,\frac{d}{b})=0$. Hence,
$\phi_5(t,\frac{d}{b},\frac{d}{b},\frac{d}{b})=\exp\{\frac{-\delta(a_1+a_2)}{b}\int_0^t S(t-u)du\}.$
As a special case if we take $S(t)=Ae^{(\delta)t}$, then $\int_0^t S(t-u)du=A\int_0^t e^{\delta(t-u)}du=A[\frac{e^{\delta t}-1}{\delta}]$. Hence
$\phi_5(t,\frac{d}{b},\frac{d}{b},\frac{d}{b})=\exp\{\frac{-A(a_1+a_2)}{b}(e^{\delta t}-1)\}.$
Thus at equilibrium point $E_5(\frac{d}{b},\frac{d}{b},\frac{d}{b})$, if $\delta>0$, then the probability that single resistance will persist in independent on δ .

(vi) The probability generating function at $E_6(\frac{d}{b},\frac{b+a_2}{b},\frac{d}{b})$is
$\phi_6(t,\frac{d}{b},\frac{b+a_2}{b},\frac{d}{b})=\exp\{I_1(t)+\alpha_1 I_2(a_1,x)+\alpha_2 I_2(a_2,y)\}$,

Wher $I_1(t)=\frac{-\delta(a_1+a_2)}{b}\int_0^t S(t-u)du,\ I_2(a_1,\frac{d}{b})=0$ and $I_2(a_2,y)=\frac{(\delta-a_2)}{b}\int_0^t S(t-u)du.$ Now, if we consider the case $S(t)=Ae^{(\delta)t}$, then $I_2(a_2,\frac{b+a_2}{b})=\frac{(b+a_2)}{b\delta}A[e^{(\delta)t}-1]$. It follows from the definition of probability generating function , that
$\phi_6(t,\frac{d}{b},\frac{b+a_2}{b},\frac{d}{b})=\exp\{\frac{-a_1\delta+a_2\alpha_2}{b\delta}A(e^{\delta t}-1)\}.$
Thus at equilibrium point $E_6(\frac{d}{b},\frac{b+a_2}{b},\frac{d}{b})$,if $\delta>0$ and $-\alpha_1\delta+a_2\alpha_2<0$ then the probability that single resistance will persist in is independent on δ.

(vii) The probability generating function at $E_7(\frac{b+a_1}{b},\frac{d}{b},\frac{d}{b})$is
$\phi_7(t,\frac{b+a_1}{b},\frac{d}{b},\frac{d}{b})=\exp\{I_1(t)+\alpha_1 I_2(a_1,x)+\alpha_2 I_2(a_2,y)\}$,

where $I_1(t)=\frac{-\delta(a_1+a_2)}{b}\int_0^t S(t-u)du,$
$I_2(a_2,\frac{d}{b})=0$ and $I_2(a_1,x_1)=\frac{(\delta+a_1)}{b}\int_0^t S(t-u)du.$
Now , if we consider the special case $S(t)=Ae^{(\delta)t}$, then, $I_2(a_1,\frac{b+a_1}{b})=\frac{(b+a_1)}{b\delta}A[e^{(\delta)t}-1]$. Hence
$\phi_7(t,\frac{b+a_1}{b},\frac{d}{b},\frac{d}{b})=\exp\{\frac{-a_2b+a_1a_2}{b\delta}A(e^{\delta t}-1)\}.$

Thus at equilibrium point $E_7(\frac{b+a_1}{b},\frac{d}{b},\frac{d}{b})$,if $\delta > 0$ and $-\alpha_2\delta + \alpha_1\alpha_2 < 0$, then the probability that single resistance will persist in is independent on δ .

(viii) The probability generating function at $E_8(\frac{b+a_1}{b},\frac{b+a_2}{b},\frac{d}{b})$

$\phi_8(t,\frac{b+a_1}{b},\frac{b+a_2}{b},\frac{d}{b}) = \exp\{I_1(t) + \alpha_1 I_2(a_1,x) + \alpha_2 I_2(a_2,y)\}$,

where $I_1(t) = \frac{-\delta(a_1+a_2)}{b}\int_0^t S(t-u)du$, and $I_2(a_1,\frac{b+a_1}{b}) = \frac{(\delta+a_1)}{b}\int_0^t S(t-u)du$. Now , if we consider the case $S(t) = Ae^{(\delta)t}$, then $I_2(a_1,\frac{b+a_1}{b}) = \frac{(\delta+a_1)}{b\delta}A[e^{(\delta)t} - 1]$ and $I_2(a_2,\frac{b+a_2}{b}) = \frac{(\delta+a_2)}{b}\int_0^t S(t-u)du$. Now , if we consider the special case $S(t) = Ae^{(\delta)t}$, then, $I_2(a_2,\frac{b+a_2}{b}) = \frac{(\delta+a_2)}{b\delta}A[e^{(\delta)t} - 1]$. Hence from the definition of probability generating function , we get

$\phi_8(z,\frac{b+a_1}{b},\frac{b+a_2}{b},\frac{d}{b}) = \exp\{\frac{a_1\alpha_1+a_2\alpha_2}{b\delta}A(e^{\delta t} - 1)\}$.

Thus at equilibrium point $E_8(\frac{b+a_1}{b},\frac{b+a_2}{b},\frac{d}{b})$, if $\delta > 0$, then the probability that single resistance will persist in is independent on δ .

4. Conclusion

In this paper, we study the behavior of solutions of permanent drug resistance model. We established the existence of possible eight equilibria of the form $E_1 = (1,1,1)$, $E_2 = \left(\frac{a_1+d}{b},1,1\right)$, $E_3 = \left(1,\frac{a_2+d}{b},1\right)$, $E_4 = \left(\frac{a_1+d}{b},\frac{a_2+d}{b},1\right)$, $E_5 = \left(\frac{d}{b},\frac{d}{b},\frac{d}{b}\right)$, $E_6 = \left(\frac{d}{b},\frac{a_2+b}{b},\frac{d}{b}\right)$, $E_7 = \left(\frac{a_1+b}{b},\frac{d}{b},\frac{d}{b}\right)$ and $E_8 = \left(\frac{a_1+b}{b},\frac{a_2+b}{b},\frac{d}{b}\right)$. We introduced condtions for local stability and instability of all equilibria in Proposition 2.1. We also discussed the global stability using Lozinski measure. We deduced in Theorem 2.2 that all solutions of (1.5) which initiate in R^3 are uniformly bounded. Then we used Bendixon-Dulac Theorem to show that system (2.5) does not have nontrivial periodic orbits. The probability generating function $\phi(t,x,y,z)$ for the two drugs resistance model in all possible cases of the parameters b, d, α_1 and $\alpha_2$is calculated. We used these probabilities to calculate the probability that resistance is generated after treatment. Our obtained results improve and partially generalize those obtained in [9]-[14].

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Body Mass Index as an Assessment Tool for Overweight and Obesity in School Children in El-Qalubia Governorate

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Abstract: Aim of the study was to design BMI (Body Mass Index) charts to the studied group, to determine the prevalence of overweight and obesity in the group and to provide suitable recommendations for prevention overweight and obesity. Methods: This was a cross sectional study that included 500 students collected from primary and preparatory schools, aged 7-15 years and living in El-Qalubia governorate through the academic year 2010/2011. A self administered questionnaire was used; it included some socio-demographic characteristics and measurements for weight and height of students. Data was collected, revised and entered using the statistical package SPSS. Results: Obese students represented 20.4% of students. Obesity increased in younger students with mean age 9.33±2.094 years and increased in male students (55% of all obese students), while overweight increased in female students (54.5% of all overweight students). Female students recorded higher values over males in weight during the first 5 years of age (7-11 years old) then, boys become heavier than girls. Moreover, there was a gradual increase in height of female and male students at age 7-15 years old. In addition, the current study indicated that female students have the higher values of BMI from age 7 to 15 years old than males.

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Keywords: BMI (kg/m^2), obesity, and overweight.

1. Introduction

The increasing prevalence of overweight and obesity in childhood poses an ever-increasing problem. Since childhood, overweight affects both the children's health and their social integration and these children tend to become obese adults **(Reinehr et al., 2010).**

The recent rapid increase in childhood overweight and obesity are highly attributed to the modern obesogenic environment. Insufficient physical activity, changes in dietary habits and sociodemographic and environmental factors have been widely associated with higher weight in children and adults **(Marja et al., 2009).**

In a report from the Egyptian National Nutrition Institute, there is increasing prevalence of obesity among children and adolescents reaching 5.8% among males and 9.7% among females **(Abul Magd, 2009).**

Programmes of obesity prevention in schoolchildren should promote healthy eating patterns, especially a decrease in caloric intake, and enhanced physical activity, including reduced sedentary behaviour and adequate opportunities to pursue a more active lifestyle **(Zellner et al., 2007).**

Subjective assessment of childhood overweight and obesity has been shown in both clinical practice and publication to be inadequate, and therefore we need to perform objective assessment. Weight itself is an inadequate measure, given its relation to height, but body mass index (BMI) has been shown in childhood not only to screen for excess fatness, but also to be related to morbidity and it meets the following criteria: the simplicity of the measure, the cost, ease of use, and acceptability to the subjects **(SING,2003).**

The body mass index (BMI) is calculated from a person's weight in kilograms and height in meters (kg/m2). Childhood overweight and obesity are identified by using the international cut-off points, based on average centiles estimated to pass through BMI values **(Cole et al., 2000).**

BMI is an excellent choice of instrument for the definition of childhood overweight and obesity, as it changes with age and differs between sexes, so age and sex- specific centile charts are needed for childhood, with use of cut-off levels. There is widespread support for the use of BMI in childhood **(SING, 2003).**

Although obesity is caused by both genetic and environmental factors, the changes in environmental factors are likely to cause a more change in BMI percentiles. However, the change of the BMI distribution raises the question of whether some individuals are genetically more susceptible to changes in these environmental factors than others are **(Kurokawa et al., 2009).**

Overweight and obese children are at increases risk of a range of medical conditions affecting cardiovascular health (hypertension, hypercholesterolemia), the endocrine system (insulin resistance, impaired glucose tolerance hyperinsulinism, menstrual irregularity, type 2 diabetes mellitus), the pulmonary system (asthma, obstructive sleep apnea syndrome), the musculoskeletal system (genu varum, slipped capital femoral epiphysis), and mental health (depression, low self-esteem, distorted body image, eating disorders) **(Lobstein et al.,2004).**

2. Subjects and Methods:

The present work was a cross-sectional study.

Sample

Random sampling of students aged 7-15 years with a total number of 500 students of both sexes was included in the study (primary and preparatory schools), living in El-Qalubia governorate through the academic year 2010/2011. The students of the study were collected from schools in urban and rural areas.

Data collection

The data were collected by using a semi structured, self-administrated questionnaire guided by researcher's instructions. The questionnaire included socio-demographic characteristics and measurements for weight and height of students. Students were weighed to the nearest one kg, lightly dressed and barefooted. Standing height was measured to the nearest one cm, with shoes off, feet together and head in the Frankfort horizontal plane **(Tokmakidis et al., 2007).** Body mass index (BMI) was calculated as: Body weight (Kg)/ $[height(m)]^2$. Overweight and obesity were identified using age and gender specific international cut-off points, based on average centiles estimated to pass through BMI values **(Cole et al., 2000).**

Table (1): The international cut off points for definition of overweight and obesity (Cole et al., 2000).

BMI cut-off values / Age (years)	Overweight		Obese	
	Males	Females	Males	Females
7	17.92	17.75	20.63	20.51
8	18.44	18.35	21.60	21.57
9	19.10	19.07	22.77	22.81
10	19.84	19.86	24.00	24.11
11	20.55	20.74	25.10	25.42
12	21.22	21.68	26.02	26.67
13	21.91	22.58	26.84	27.76
14	22.62	23.34	27.63	28.57
15	23.29	23.94	28.30	29.11

The socio-demographic data for all students was considered regarding; the age, sex, residence, monthly income of family **(Modified from Fahmi and El-Sherbini, 1983).**

Data were collected, revised and analyzed.

Statistical analysis

Data were collected, revised and entered using the statistical package SPSS. The collected data was tabulated and analyzed with the suitable statistical methods using mean value ± standard deviation, T-test, analysis of variant and chi square test. BMI was calculated for each age and sex separately. The mean values and standard deviation were determined for each age and sex, and graphically smoothed. P value of less than 0.05 was considered statistically significant.

3. Results

Normal weight students were 42.2% while overweight students were 37.4% and the obese students were 20.4%. As regard age, this study revealed that obesity increased in younger students with mean age 9.33±2.094 years. More over obesity increased in male students (55% of all obese students), while overweight increased in female students (54.5% of all overweight students).

Regarding weight, female students recorded higher values over males in weight girls during the first 5 years of age (7-11 years old) then, boys become heavier than girls. Moreover, there was gradual increase of height of female and male students at age 7-15 years old. In addition, the current study indicated that female students have the higher values of BMI from age 7 to 15 years than males (Table 1).

Table 2 illustrated that the prevalence of the students of normal weight was 42.2% while that of overweight was 37.4% and that of the obese students was 20.4%. And figure (1) revealed such distribution.

Table 3 illustrated that obesity increased in younger students with mean age 9.33±2.094 years with a highly significant relation between groups.

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Table (2): Prevalence of normal weight, overweight and obesity in the studied group.

The groups	No.	%
Normal weight	211	42.2
Over weight	187	37.4
Obese	102	20.4
Total	500	100.0

Figure (1): Prevalence of normal weight, overweight and obesity in the studied group.

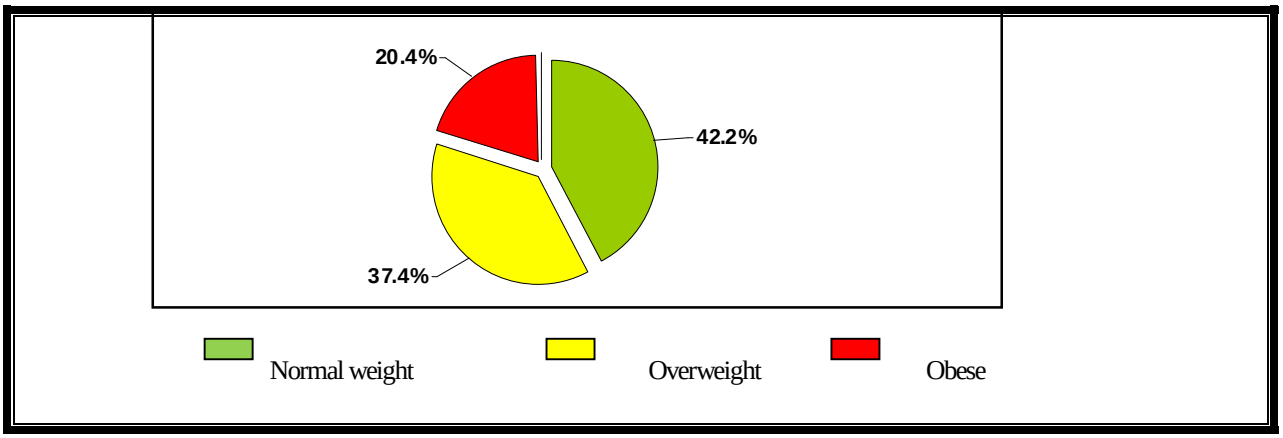


Table (3): The mean values of age for the groups

Age (year)	The groups			f	p	Between groups
	Normal weight (n=211)	Overweight (n=187)	Obese (n=102)			
Range	7-15	7-15	7-15	37.4	<0.001	P1<0.05
Mean	11.16	11.83	9.33			P2<0.05
±S.D	2.584	2.225	2.094			P3<0.05

P1 = normal and overweight P2 = normal and obesity P3 = overweight and obesity

Table (4): Distribution of the studied group according to sex

Sex	The groups						Total	
	Normal weight		Overweight		Obese			
	No.	%	No.	%	No.	%	No.	%
Male	108	43.40	85	34.10	56	22.50	249	100.0
Female	103	41.00	102	40.70	46	18.30	251	100.0
Total	211	42.20	187	37.40	102	20.40	500	100.0

X^2=2.6 **P**>0.05

Figure (2): Distribution of the studied group according to sex

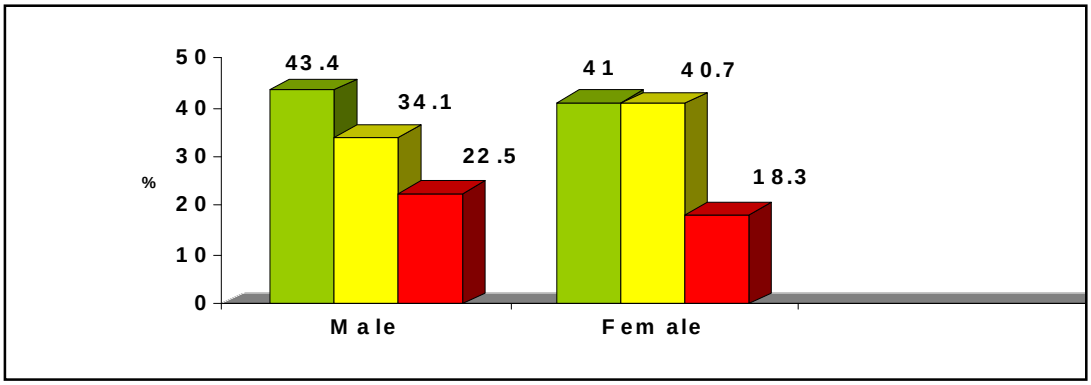


Table 4 illustrated that obesity increased in male students (55% of all obese students), while overweight increased in female students (54.5% of all overweight students). And that was statistically insignificant. And figure (2) revealed such distribution

Table (5): Distribution of the studied group according to residence

Residence	The groups						Total	
	Normal weight		Overweight		Obese			
	No.	%	No.	%	No.	%	No.	%
Urban	99	40.6	58	23.8	87	35.6	244	100.0
Rural	112	43.7	129	50.4	15	5.9	256	100.0
Total	211	42.20	187	37.40	102	20.40	500	100.0

$X^2 = 78.4$ $P < 0.001$

Table 5 illustrated that obesity increased in urban students (85% of all obese students) while overweight increased in rural students (69% of all overweight students) with a highly significance.

Table (6): The mean values of weight (kg) for female and male students according to the age in the studied group.

Weight (kg) / Ages	Female		Male	
	Mean	Standard deviation	Mean	Standard deviation
7 years	25.68	5.61	24.13	5.01
8 years	30.85	7.74	28.35	6.49
9 years	31.40	9.57	34.25	10.61
10 years	36.39	10.10	32.72	8.63
11 years	38.14	7.78	36.75	7.74
12 years	47.55	12.40	45.30	9.43
13 years	48.67	8.35	50.02	7.83
14 years	49.03	7.15	45.86	7.80
15 years	47.10	11.30	51.85	11.80

This table illustrated that female students recorded higher values over males in weight during the first 5 years of age (7-11 years old) then, boys become heavier than girls. And figure (3 & 4) revealed that.

Figure (3): The mean values of weight (kg) for female according to the age in the studied group.

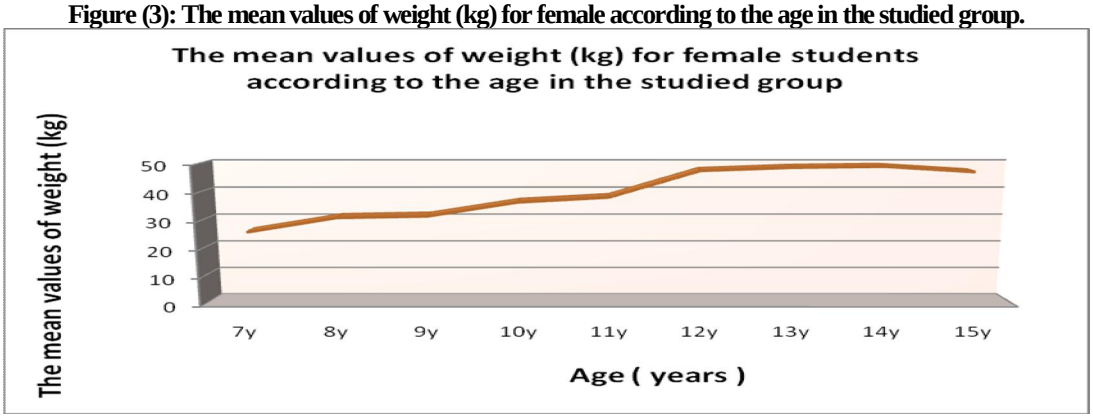


Figure (4): The mean values of weight (kg) for male according to the age in the studied group.

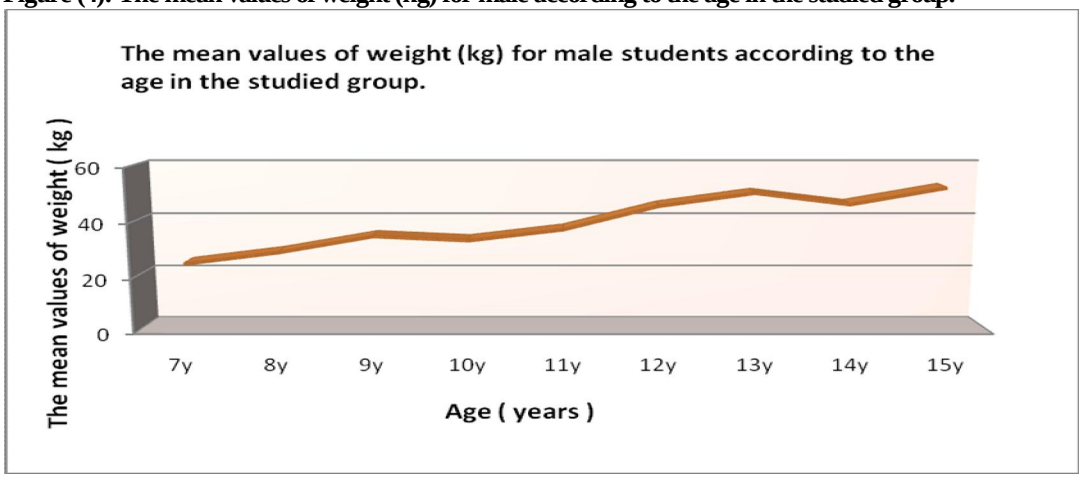


Table (7): The mean values of height (cm) for female and male students according to the age in the studied group.

Height (cm) / Ages	Female		Male	
	Mean	Standard deviation	Mean	Standard deviation
7 years	111	7.9	113	5.7
8 years	120	6.1	117	7.4
9 years	121	8.9	122	7.6
10 years	127	8.3	125	8.8
11 years	135	7.5	133	9.6
12 years	141	7.1	141	7.5
13 years	145	6.4	145	6.7
14 years	146	5.3	145	7.4
15 years	150	7.5	151	7.8

This table illustrated that there was a gradual increase of height of female and male students at age 7-15 years old. And figure (5 & 6) revealed that.

Figure (5): The mean values of height (cm) for female according to the age in the studied group.

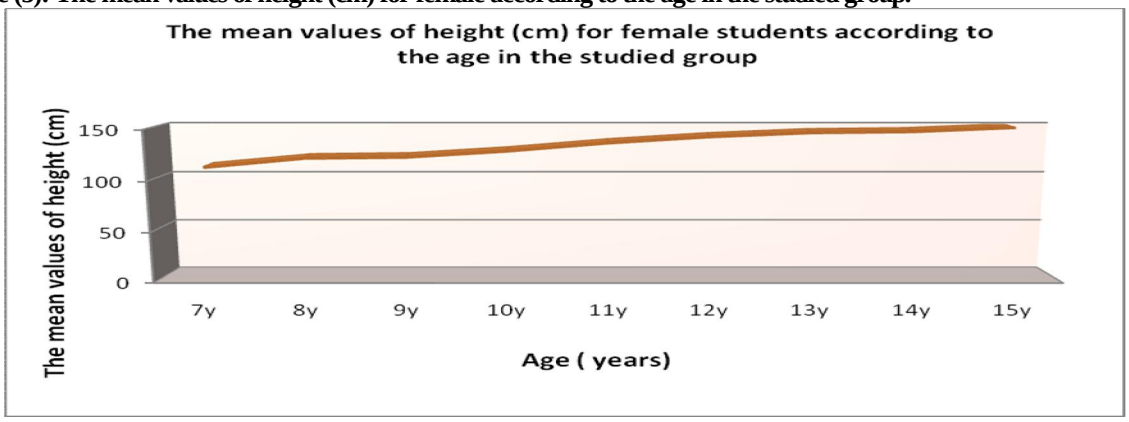


Figure (6): The mean values of height (cm) for male according to the age in the studied group.

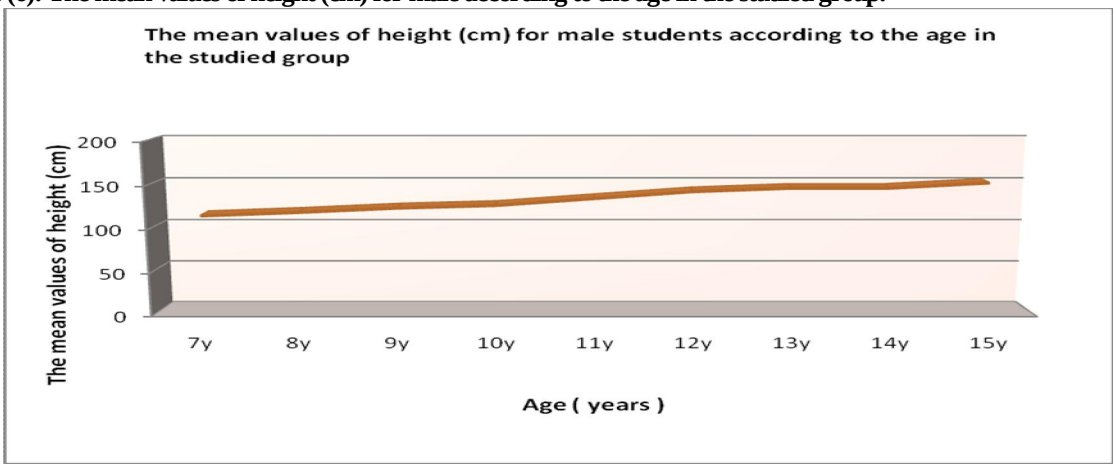


Table (8): The mean values of BMI (kg/m^2) for female and students according to the age in the studied group.

BMI / Ages	Female		Male	
	Mean	Standard deviation	Mean	Standard deviation
7 years	21.2	5.4	18.9	3.7
8 years	21.4	5.2	20.9	5.1
9 years	21.3	4.6	22.7	5.5
10 years	22.9	8.3	21.0	4.6
11 years	20.9	3.1	20.7	3.5
12 years	23.5	4.7	23.0	4.8
13 years	23.1	3.0	23.9	3.2
14 years	22.9	2.7	21.7	3.4
15 years	20.9	3.4	22.8	5.3

This table illustrated that female students have the higher values of BMI from age 7 to 15 years than males. And figure (7 & 8) revealed that.

Figure (7): The mean values of BMI (kg/m^2) for female according to the age in the studied group.

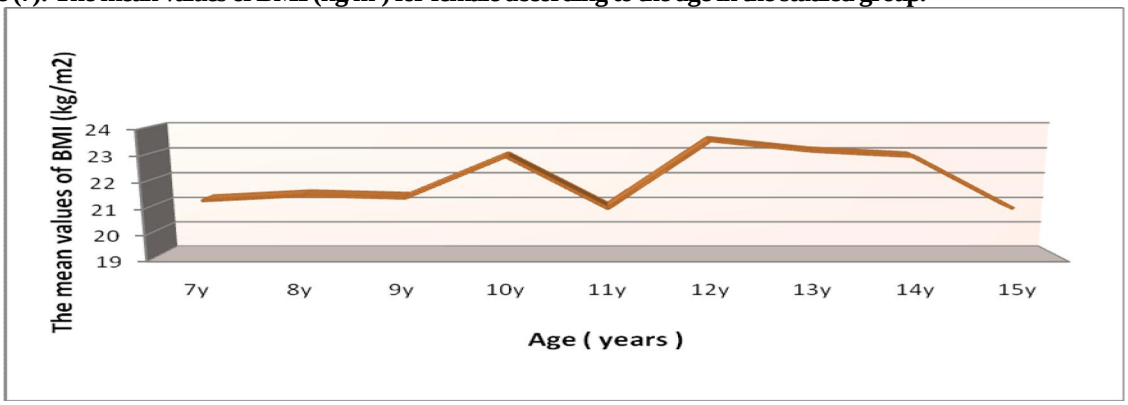


Figure (8): The mean values of BMI (kg/m^2) for male according to the age in the studied group.

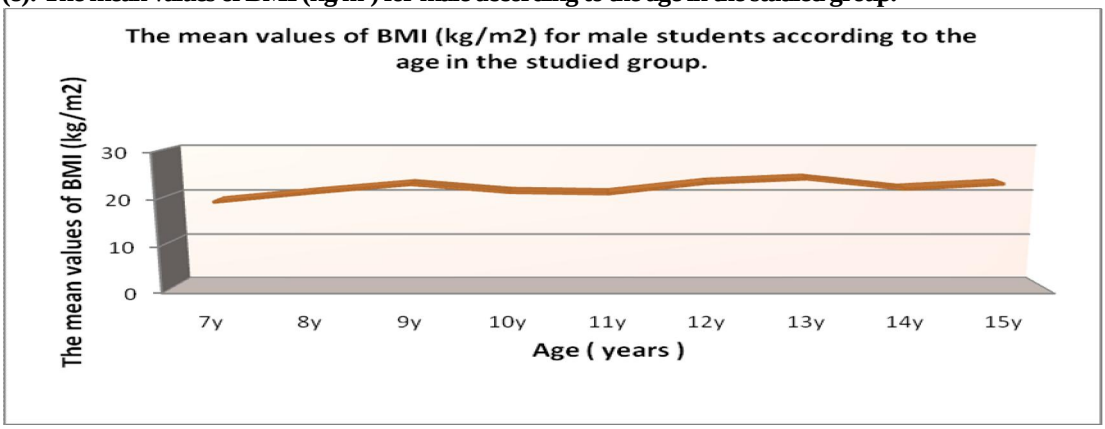


Table (9): The mean values of the monthly income of the family (L.E.) for the groups .

Monthly income of the family (L.E.)	**The groups**			f	p	Between groups
	Normal weight (n=211)	Overweight (n=187)	Obese (n=102)			
Range	250-2100	100-2000	400-2500	6.3	<0.05	P1>0.05
Mean	863.46	809.36	954.80			P2<0.05
±S.D	326.121	298.477	396.464			P3<0.05

P1 = normal and overweight P2 = normal and obesity P3 = overweight and obesity

This table illustrated that the mean values of monthly income for the normal subjects was 863.46±326.12 LE, for the overweight subjects it was 809.36±298.47 LE and for the obese subjects it was 954.8±396.46 LE. This was statistically significant.

Table (10): Correlation between monthly income (L.E.) and BMI (kg/m^2)

	Monthly income	
	r	p
BMI (kg/m^2)	0.1	>0.05

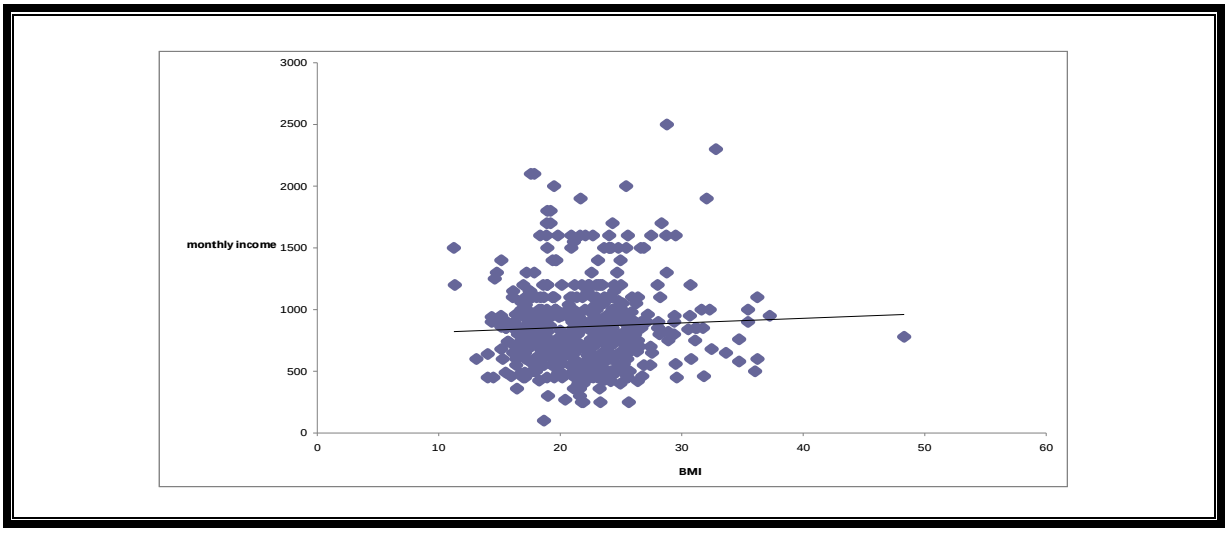
This table illustrated that there was a positive correlation between monthly income (L.E.) of the family and BMI (kg/m^2) of the students which was statistically insignificant and figure (9) revealed these results.

Table (11): Distribution of the studied group according to social class.

Social class	**The groups**						**Total**	
	normal weight		overweight		obese			
	No.	%	No.	%	No.	%	No.	%
High social class	147	42.9	127	37.0	69	20.1	343	100.0
middle social class	59	43.1	49	35.7	29	21.2	137	100.0
Low social class	5	25.0	11	55.0	4	20.0	20	100.0
Total	211	42.2	187	37.4	102	20.4	500	100.0

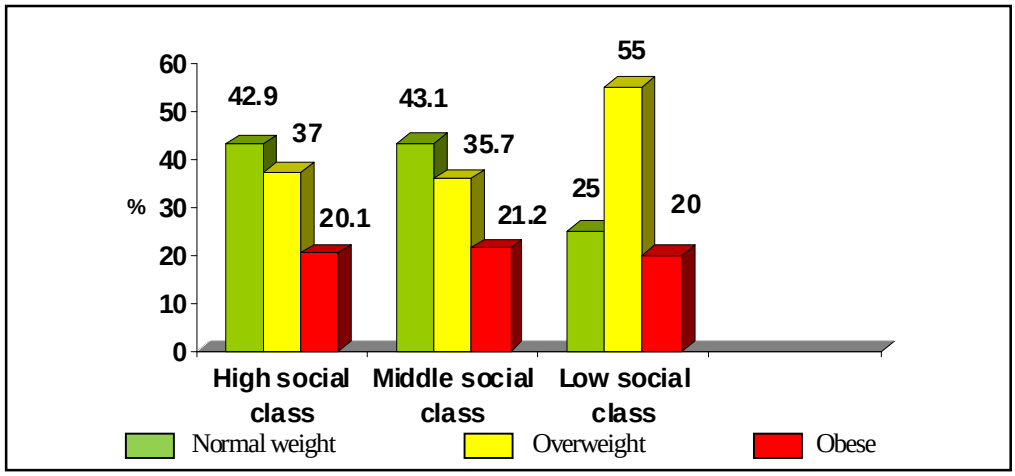
X^2=3.3 **P**>0.05

Figure (9): Correlation between monthly income (L.E.) and BMI (kg/m^2)



This table illustrated that the percentage for distribution of the studied group in subjects of high social class was 42.9% of the normal weight, 37.0% of the overweight and 20.1% of the obese. The percentage for distribution of the studied group in subjects of middle social class was 43.1% of the normal weight, 35.7% of the overweight and 21.2% of the obese. The percentage for distribution of the studied group in subjects of low social class was 25.0% of the normal weight, 55.0% of the overweight and 20.0% of the obese. More than two thirds of obese students belonged to high social class families. This was statistically insignificant and figure (10) revealed such distribution.

Figure (10): The distribution of the studied group according to social class.



Findings from the present study indicated that obesity increased in urban students (85% of all obese students) while overweight increased in rural students (69% of all overweight students).

The current work revealed that the mean monthly income for the normal subjects was 863.46±326.12 LE, for the overweight subjects it was 809.36±298.47 LE and for the obese subjects it was 954.8±396.46 LE. More than two thirds of obese students belonged to high social class families.

4. Discussion:

Obesity in children has increased dramatically in recent years. In many developed countries childhood overweight and obesity has reached epidemic proportions, as these has been doubled in the past 20 years **(Hedley et al., 2004).** Among children aged 5 to 17 years, about 155 million are estimated to be overweight; 30 to 45 million of them are classified as obese **(Lobstein et al., 2004).**

This study was conducted to reveal the prevalence of overweight and obese children among the

studied group at the governmental schools at El-QALUBIA, using the body mass index and the international cut off points for definition of overweight, and obesity **(Cole et al,. 2000).**

It was a cross sectional short-term study that was conducted on female and male students of the governmental schools at El-QALUBIA during the academic year 2010/2011. The data were collected from participants and their parents with the help of simple self-administered questions as well as weight and height measurement.

This study comprised 500 students. Their parents were consented for the study after explaining its purpose and method and after exclusion of those who refuse or were absent from the school.

The overall prevalence of overweight subjects was 37.4%. Moreover, it was for the obese subjects 20.4%. These results were different from that among Menoufiya and Assuit female students which were (44.4% and 14%) for overweight and (13.4% and 7.8%) for obese respectively **(Farahat and Abou El-Fath, 2001).** Possibly, this was because the difference in sex of the students in both studies.

As when the age of the children increases, the interest of their bodies' forms increases; it was found in the current study that the mean age of the normal weight subjects was 11.16±2.584 years and 11.83±2.225 years for the overweight subjects while it was 9.33±2.094 years for the obese subjects. These findings were confirmed by **Chrzanowska et al., (2007)** who found that the greatest increase in BMI was in the youngest age groups (7–12 years for boys and 7–10 years for girls).

Salem et al., (2002) found that the prevalence of obesity among Egyptian children was 14.7% and 15.08% for males and females respectively, their age ranged from 12-18 years. These finding did not agree with results in the current work which revealed that prevalence of obesity among the studied group was 22.5% for males and 18.3% for females. The difference in age group between the students in both studies might be the cause of the different results.

As regard weight of female students, the current study revealed that there was acute increase of weight at age 7-10 years old followed by gradual increase at age of 10- 14 years then decreased at the age of 15.

As regard weight of male students the current study revealed that there was gradual increase of weight at age 7-15 years old. Female students recorded higher values over males in weight except at the ages of 13 and 15 years boys were heavier than girls. This was not in agreement with **Yamamah et al., (2010)** who found that boys recorded higher values in weight over girls during the first 8years of age, after that, girls have higher values than boys at ages 9 to 14. This difference might be due to the small sample size of the current work.

As regard height of female and male students the current study revealed that there was gradual increase of height at age 7-15 years old.

Regarding BMI, female students have the higher values of BMI than males except at the ages of 9, 13 and 15 years boys have the higher values than girls. These results were confirmed by **Yamamah et al., (2010)** who found the same results in their study 5245 healthy children in South Sinai aged 9-16 years old.

As regard BMI of female students the current study revealed that there was gradual increase of BMI at age 7-10 years old followed by slightly decrease at age of 11 years then re-increased at the age of 12 and 13years finally it decreased again at the age of 14 and 15 years. These changes might be due to growth spurt in this age but at the age of 14 and 15 the interest of body shape of girls affected their weight so they decreased their weight for beauty.

As regard BMI for male students the current study revealed that there was rapid increase of BMI at age 7-9 years old followed by rapid decrease at age of 10 and 11 years then more rapid re-increased at the age of 12 and 13years finally it gradually decreased again at the age of 14 and 15 years. These changes might be due to growth spurt in this age but at the age of 14 and 15, the height of males increase more than weight leading to decrease BMI and that was revealed from the current study as height affected BMI more than weight.

In developing countries, the transition from rural agrarian to urban economies has accelerated the appearance of the obesity, which is accompanied by shift in overall health burden from infectious disease and under nutrition to western chronic diseases such as cardiovascular disease, cancer and diabetes **(Oken and Gillman, 2003).**

That appeared in the current work as it was found that about one third of urban students were obese while only few percent (5.9%) of rural students were obese. This is in agreement with **Martinez (2006)** and who found that obesity was less prevalent in rural adolescents as rural life style and dietary habits there, may be contributing factors to adjust weight among rural adolescents.

As revealed from the current work, the comparison regarding to the residence for the overweight subjects it was found that about one fifth of urban students were overweight while one half of rural students were overweight. **Jackson et al., (2003)** did not report the same results as he said" In a study among female adolescents in Egypt overweight was more prevalent in urban girls than in rural girls".

As some socio-economic factors influence quality and quantity of consumed food, through

varied income, food habits and life style **(Khalil, 1999),** the current work revealed that the mean monthly income for the normal subjects was 863.46±326.12 LE , for the overweight subjects it was 809.36±298.47 LE and for the obese subjects it was 954.8±396.46 LE. This is not in agreement with **Canoy and Buchan (2007)** and **Moreira and Padra (2006)** who found that obesity depends on the stage of economic development as it increases in low-income countries and decreases in high-income countries. This could be attributed to that the difference in social characters of the studied groups. As when monthly income in the western countries increases, health-orientation and nutritional regimen following increase, leading to decrease the weight. On the contrary in eastern countries, high monthly income increase food consumption leading to increase the weight.

While **Fernald (2007)** found that the highest social group had significantly higher BMI when compared with other groups, the current study did not report any significant relation between social class and obesity. This could be attributed to that in the current study students who belonged to low social class were only 4.0% while those belonged to high social class were 68.6%.

Recommendations:

I- Parents:

Educate parents about:

1- Hazards of obesity in childhood.

2- Importance of making healthy foods easily available to the child and serve these foods in positive mealtime situations in order to help their child develop healthy food habits.

3- Developing the sense of trust and attachment to provide adequate physical, emotional and nutritional care for obese especially those complaining of complications.

4- Encouraging children to participate in physical activities to follow diet control especially for fat, refined sugar and sport drink and weight control.

5- Integrating physical activity into daily life, not just to increase leisure time exercise.

II - Schools:

1- Integrating adequate information about healthy life and importance of monitoring weight regularly within school curriculum.

2- As overweight and obese students are at risk of being school underachievers, hence, the construction of special classes in regular schools or special schools for those with profound handicapping. School playgrounds should be constructed, bearing in mind the needs of obese. School curriculum must provide adequate information about healthy life and importance of monitoring weight regularly.

3- Adequate sessions for physical exercises should be stressed up on in school for proper physical and psychological fitness.

4- Educate students not to practice bad behavior and try to avoid or correct sedentary life style.

5- Proper understanding the concept of school insurance that has been emerged to ease the financial burden of medical care and to optimize the use of available health services.

6- Weight monitoring and weight control should be periodically checked for school students even without periodical examination.

7- School nurse has the duty of supervision of required drugs especially for that drugs which must be provided daily in co-ordination with home.

III- Role of NGOs (Non Governmental Organizations):

Increase in the number of constructed youth clubs and to provide these clubs with all facilities for the welfare of all students, bearing in mind the needs of overweight and obese students.

IV-Government:

1-The Ministry of Health should stress on the presence of Nutrition labels on fast-food packing.

2- The Ministry of Health should provide continuous medical education for general practitioners, nurses and specialist to upgrade their knowledge about:

- The magnitude of the problem.
- Risk factors and complications.

-Ways of prevention and prope management.

-Health care of all students and better case finding to prevent the occurrence of related complications.

3- The supreme council for youth and sports has the duty of proper physical and mental states for students in general and overweight and obese in particular.

Conclusion:

It could be concluded from this study that obesity among both primary and preparatory school children is a prevalent problem in the studied group in El-Qalubia governorate, with a tendency to badly affect the children, so, it is suggested that research efforts should focus more on this area with emphasize on intervention for prevention. This study recommended another researches should be done on larger sample size and more different places in El-Qalubia governorate and the other governorates to compare the results with each other.

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Comparative Histological and Ultrastructural Studies on the Stomach of *Schilbe mystus* and the Intestinal Swelling of *Labeo niloticus*

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Abstract: The present investigation aims to illustrate the histological and ultrastructural differences of the stomach of a carnivorous fish, *Schilbe mystus,* and the intestinal swelling of a herbivorous fish, *Labeo niloticus.* The stomach of *Schilbe mystus* is morphologically divided into three portions: the cardiac, pyloric and fundic portions. However, the histological examination revealed that the stomach is actually divided into two portions: the cardio-fundic portion and the pyloric one. Moreover, the mucosa of the cardiofundic portion of the stomach of *Schilbe mystus* revealed that it consists of two types of cells: the luminal and glandular cells. The luminal cells, which line the gastric lumen and gastric pits, are tall columnar with apically concentrated secretory granules that have a positive PAS reaction. In addition, the glandular cells, which line the bodies of the gastric glands, are pyramidal in shape and possess scattered secretory granules. The pyloric portion of the stomach has the same structure as the cardio-fundic portion, but the gastric glands are completely absent. The histological examination of the mucosa of the intestinal swelling of *Labeo niloticus* revealed that it is built up of a simple columnar epithelium that contains goblet cells. The ultrastructural examination of the gastric mucosa of the stomach of *Schilbe mystus* revealed three types of cells: the luminal, endocrine and exocrine (oxynticopeptic) cells. The luminal cells are tall columnar and are characterized by the presence of apically aggregated secretory granules and a prominent vesiculotubular system. The gastric endocrine cells are mainly pyramidal in shape and are characterized by numerous secretory granules, which exhibit various sizes and shapes. According to the dominant size of the secretory granules, the endocrine cells are differentiated into three types: type-I, type-2 and type-3 cells. Moreover, the gastric glands of *Schilbe mystus* are made up of a single type of exocrine cells, the oxynticopeptic cells. The oxynticopeptic cells are characterized by the combined features of the mammalian oxyntic and peptic cells. The oxynticopeptic cells have numerous apical microvilli, a well-developed vesiculotubular system, numerous mitochondria, a rough endoplasmic reticulum and numerous secretory granules.The ultrastructural examination of the mucosa of the intestinal swelling of *Labeo ni/oticus* revealed that the enterocytes are tall columnar and possess welldeveloped microvilli, numerous mitochondria, microtubules, a few endocytotic channels and aggregated chylomicrons.

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1. Introduction

AI-Hussaini and Kholy (1953) found that the stomach of *Tilapia nilotiea* is divided into a wide caecal region and a narrow pyloric one. They believed that the oxyntic and peptic cells are not found in the gastric mucosa of some omnivorous teleosts. In addition, Kapoor *et al.* (1975) and Grau *et al.* (1992) stated that the main features of the carnivorous fish digestive tract are the presence of a large stomach and a short intestine.

Reifel and Travill (1978) stated that the shape of the teleostean stomach is variable. They reported that the stomach shape may be rectilinear as in *Esox amerieanus* and *Esox lucius* or siphonal as in *Ambloplites rupestris, Lepomis maerochirus* and *Pomoxis nigromaculatus* or caecal as in *Ictalurus nebulosus, Mieropterus salmoides* and *Pereajlaveseens.* They also added that the rectilinear and caecal types are associated with piscivorous species.

Noaillac-Depeyre and Gas (1982) stated that the gastric mucosa of the perch, *Perea jluviatilis,* and the catfish, *Ameiurus nebulosus,* contains three and four different types of endocrine cells, respectively. Each endocrine cell type possesses characteristic secretory granules. They also demonstrated that these cells are mainly found among the mucoid cells and rarely among the gastric glandular cells.

Klumpp and Nichols (1983) found that the absence of the stomach in the alimentary tract of the herbivore southern sea garfish, *Hyporhamphus melanoehir,* indicate that acid hydrolysis does not have a role in the breakdown of the plant cell walls. Therefore, they suggested that this fish rely on the action of its pharyngeal mill to rupture the plant cell walls.

Martin and Blaber (1984) stated that the stomach of a caecal-type in some Ambassidae fish is described in fish with predatory habits and might serve as a storage site for irregular intake of large quantities of food.

Cataldi *et al.* (1987) mentioned that the gastric glands, which contain one type f cells, are present in the cardiac region and increase in number in the fundic one, but they are completely absent in the pyloric region.

MacDonald (1987), in the juvenile and adult Dover sole, *Solea solea,* and Caceci and Hrubec (1989 & 1990), in the black mollie, *Poeeilia* spp., stated that the apical microvilli characterize the epithelial cells of all regions of the gastrointestinal tract. Also, Osman and Caceci (1991) observed that the gastric epithelium of *Tilapia nilotiea* has numerous short apical microvilli.

Tibbetts (1997) stated that the digestive system of the snub-nosed garfish, *Arrhamphus sclerolepis krefftii,* lacks many of the alimentary modifications common in the alimentary tract of the herbivorous fishes such as the acidic stomach, long intestine and pyloric caeca.

Arellano *et al.* (2001) stated that the gastric glands of *Solea senegalensis* are numerous in the pyloric and fundic regions, but they are absent in the cardiac portion. These glands are formed of two types of cells: the light and dark cells. Moreover, Gallagher *et al.* (2001) found that the gastric glands of the carnivorous pinfish, *Lagodon rhomboides,* have both mucous and secretory cells. They stated that the secretory cells are of a single type, which has both acid- and enzymesecreting properties.

Unal *et al.* (2001) stated that the true stomach of *Chalealburnus tarichi* is absent histologically, and instead there is an expansion at the anterior part of the intestine called the post-oesophageal swelling.

Diaz *et al.* (2003) stated that the stomach of *Engraulis anchoita* consists of two parts, the cranial and pyloric parts, both of which are lined by a simple columnar epithelium. They also added that the gastric glands, which are of the branched tubular type, are located along the stomach and are formed of a single type of glandular cells. On the contrary, Carrasson *et al.* (2006) stated that the gastric glands of *Dentex dentex,* which are of the simple tubular type, are found in the cardiac portion only and are formed of two glandular cell types.

Petri nee *et al.* (2005) stated that the presence of mucosubstances in the mucosa of the digestive tract of *Esox lucius* and *Silurus glanis* are involved in lubrication and protection processes of the mucosa against acidity and enzymatic actions.

The present investigation aims to illustrate the histological and ultrastructural differences pf the stomach of a carnivorous fish, *Schilbe mystus,* and the intestinal swellingofa herbivorous fish, *Labeo niloticus.*

2. Material and Methods

The freshwater Nile silver catfish, *Schilbe mystus* (Linnaeus, 1758), (Order: Siluriformes, Family: Schilbeidae) and the freshwater Nile carp, *Labeo niloticus* (Forsski'll, 1775), (Order: Cypriniformes, Family: Cyprinidae) were used in the present investigation.

1-Collection of the samples

The adult silver catfish, *Schilbe mystus,* ranging from 15 to 28 cm in length,

and the Nile carp, *Labeo niloticus,* ranging from 20 to 40 cm in length, were caught alive from the River Nile at different localities between Beni Suef and Giza. They were killed and dissected immediately in the field.

2- Histological and ultrastructural studies

Fresh adult specimens were carefully dissected and small pieces of the stomach and intestinal swelling of *Schilbe mystus* and *Labeo niloticus,* respectively, were fixed in aqueous Bouin's fluid. Sections of 5-7 µm thick were cut and were stained with Harris' haematoxylin and counterstained with eosin. Some sections were stained with periodic acid-Schiff reagent (PAS) and counterstained with haematoxylin to demonstrate the mucus-secreting cells.

Small pieces of the stomach and intestinal swelling of *Schilbe mystus* and *Labeo niloticus,* respectively, were immediately fixed in cold 3% glutaraldehyde solution in phosphate buffer (pH 7.2 to 7.4) or in a mixture of glutaraldehyde and formaldehyde for three hours to overnight at 4°C, then immersed in the phosphate buffer for 24 hrs.

Resin capsules were prepared and cut with the ultramicrotome into semithin sections *(*1µm thick) and stained with toluidine blue. The sections were examined with the light microscope to select the suitable areas representing the desired observations. Silver to pale golden ultrathin sections (600A in thickness) were mounted on copper grids. The sections were stained with 5% uranyle acetate and lead citrate according to Reynolds (1963). Finally, the grids were examined with a JOEL 1200 EX 11 electron microscope at the Electron Microscope Unit in the Central Laboratory, Faculty of Science, Ain Shams University and a JOEL 100S electron microscope at the Electron Microscope Unit at the National Cancer Institute.

3. Results

1-Histological studies

A-The stomach of *Schilbe mysuls*

The stomach of *Schilbe mystus* is morphologically distinguished into three portions: the cardiac, fundic and pyloric portion. However, the histological examination revealed that the cardiac and fundic portions have the same structure (Figs. 1 and 2). So, the stomach is histologically distinguished into two portions only: the anterior wide sac-like structure called the cardio-fundic portion and the posterior narrow tubular structure called the pyloric one (Fig. 3).

The wall of the stomach is generally composed of four main layers: the mucosa, submucosa, muscularis and serosa (Figs. 1, 2 and 3).

a- The cardio-fundic potion

i-The mucosa

The most important histological feature of the cardio-fundic potion of the stomach is the presence of the gastric glands in the mucosa. The mucosa of the cardio-fundic portion of the stomach is thrown up into prominent folds or rugae (Figs. 1 and 2). The gastric glands, which are of the compound tubular type, open into the gastric lumen via the gastric pits. Each gastric gland is formed of a mixed population of two types of cells: the luminal or mucus-secreting cells, and the glandular cells, which form the gland bodies (Fig. 4).

The luminal cells line the gastric lumen and the gastric pits, where the gastric glands open. These are tall columnar cells which possess homogeneous faintly stained cytoplasm and oval basally located nuclei (Fig. 4). The: apical cytoplasm of these cells contains secretory granules which have a positive PAS reaction (Fig. 5).

The glandular cells line the body of the gastric gland. They are pyramidal in shape and possess homogeneous darkly stained finely granulated cytoplasm and rounded basally located nuclei with prominent nucleoli (Fig. 4). The secretory granules of these cells have a negative PAS reaction (Fig. 5).

The lamina propria of the cardio-fundic potion of the stomach consists of loose connective tissue that holds the gastric glands and penetrates the mucosal folds (Fig. 4).

ii- The submucosa

The submucosa is formed of a loose connective tissue that lies above the lamina propria and its gastric glands (Figs. 1 and 2).

iii- The muscularis

The muscularis is well developed and consists of unstriated muscle fibres arranged in two layers: an inner circular and an outer longitudinal muscle layers (Figs. 1 and 2).

iv- The serosa

The serosa consists of a simple squamous epithelium. The cells of the serosa are small flattened with scarce homogeneous cytoplasm and compressed oval centrally located nuclei (Figs. 1 and 2).

b- The pyloric portion

i- The mucosa

The mucosa of the pyloric portion of the stomach is thrown up into prominent broad folds or rugae (Fig. 3). The mucosal epithelium is built up of simple tall columnar cells, which possess homogeneous faintly stained cytoplasm and oval, basally located darkly stained nuclei (Fig. 6). The apical cytoplasm of these cells contains concentrated secretory granules, which have a positive PAS reaction (Fig. 7). The gastric glands are totally absent.

The lamina propria of the pyloric portion of the stomach consists of a highly vascularized loose connective tissue layer, which penetrates the mucosal folds (Fig. 7). There are scattered longitudinal unstriated muscle fibres between the lamina propria and the submucosa, forming the muscularis mucosae (Fig. 3).

ii- The submucosa

The submucosa is formed of a highly vascularized loose connective tissue, which is continuous with that of the lamina propria (Fig. 3).

iii- The muscularis

The muscularis is well developed and is built up of two layers of unstriated muscle fibres: an inner circular layer and an outer longitudinal one (Fig. 3).

iv- The serosa

The serosa is a thin layer that consists of a simple squamous epithelium. The cells of the serosa are small flattened with scarce homogeneous cytoplasm and compressed oval centrally located nuclei (Fig. 3).

B- The intestinal swelling of *Labeo ni/oticus*

The wall of the intestinal swelling of *Labeo niloticus* consists of four layers: the mucosa, submucosa, muscularis and serosa (Fig. 8).

i- The mucosa

The mucosa forms numerous extremely long finger-like folds called villi. These villi possess tapering luminal ends, parallel sides and narrow crypts (Fig. 8). The mucosa is composed of a simple columnar epithelium containing scattered goblet cells. The simple columnar epithelium rests on a thin basement membrane and possesses a homogeneous finely granulated eosinophilic cytoplasm and oval nuclei with prominent nucleoli. The long axes of these nuclei are perpendicular to the basement membrane (Fig. 9).

The free borders of the columnar cells form the brush border, which is darkly stained and has a positive PAS reaction. The brush border forms a continuous layer above the columnar epithelium and

is interrupted only by the openings of the goblet cells (Fig. 9).

The goblet cells are flask-shaped with two prominent parts: the swollen apical part and the elongated basal one. Each cell opens on the luminal surface by a single pore situated at the top of the swollen part. These cells have a homogeneous faintly stained eosinophilic cytoplasm and small basally located nuclei (Fig. 9).

The lymphocytes are numerous and usually aggregate at the bases of the columnar epithelium and some of them frequently appear in the free ends of the cells.

The lymphocytes are easily detected by their scanty amount of cytoplasm and rounded darkly stained nuclei (Fig. 9).

The lamina propria consists of a loose connective tissue that penetrates and supports the villi (Fig. 8).

ii- The submucosa

The submucosa is extremely thin 'and is formed of a vascularized loose connective tissue that lies immediately above the lamina propria without a separating line (Fig. 8).

iii- The muscularis

The muscularis of the intestinal swelling consists of two muscle layers: an inner circular layer and an outer longitudinal one (Fig. 8). The muscularis of the most anterior region of the intestinal swelling is built up of striated muscle fibres (Fig. 10), while the muscularis of the posterior region is built up of unstriated muscle fibres, whose circular layer is more developed than the longitudinal one (Fig. 11).

iv- The serosa

The serosa is an extremely thin layer of simple squamous epithelium. These cells are flattened with small amount of cytoplasm and compressed oval nuclei, which are located at the widest parts of the cells (Figs. 10 and 11).

2-Ultrastructural STUDIES

A-The stomach of *Schilbe mystus*

The ultrastructural examination of the gastric mucosa of *Schilbe mystus* revealed three types of cells: the luminal (the mucus-secreting), basal endocrine and basal exocrine (the oxynticopeptic) cells. The first two types of these cells are found throughout the gastric mucosa, whereas the second type is restricted only to the cardio-fundic portion of the stomach.

i- The luminal cells

The gastric luminal cells are tall columnar (about 27 µm in average height), electron-dense and rest on a prominent basement membrane. There are numerous apically aggregated secretory granules, which are homogeneous, electron-dense and elongated in shape with variable sizes ranging from 0.16 to 0.7 µm in the long axis. Some secretory granules are frequently fused with the apical cell membrane, emptying their contents into the gastric lumen (Fig. 12).

The vesiculotubular system is made up of numerous small smooth vesicles with dense outlines. They are scattered throughout the cell cytoplasm especially at the apical region in between the secretory granules (Fig. 12). The mean diameter of these vesicles is about 0.06 µm.

The endoplasmic reticulum is differentiated into two types: the rough and smooth endoplasmic reticula. The rough endoplasmic reticulum is well developed and consists of long tubules studded with numerous ribosomes. On the other hand, the smooth endoplasmic reticulum consists of short smooth tubules. The free ribosomes are abundant and scattered throughout the cell cytoplasm (Fig. 13).

There are numerous microfilaments scattered throughout the cell cytoplasm (Fig. 13).

The nucleus is large, oval (the long axis is about 11 µm) or rounded (the diameter is about 4 µm) in shape, basally located and contains a prominent centrally located nucleolus (Fig. 12). The nuclear envelope is a double membrane consisting of an outer and an inner nuclear membrane that encompass the nucle,oplasm. The nuclear pores are found at intervals in the nuclear envelope. The chromatin is found mainly as euchromatin, which is represented by the pale granular areas of the nucleoplasm. Moreover, the heterochromatin is mainly represented by small dense granular aggregations scattered throughout the nucleus and on the inner nuclear membrane (Fig. 13).

The lateral cell membranes form numerous complex interdigitations (Fig12). Moreover, the apical ends of the lateral cell membranes of the adjacent cells join together by junctional complexes (Fig. 14).

ii- The endocrine cells

The gastric endocrine cells are electron-lucent and are scattered throughout the gastric mucosal epithelium. They are pyramidal, cuboidal or rounded in shape. These cells are seen in direct contact with the basement membrane (Fig. 15).

The rough endoplasmic reticulum consists of a network of membranous tubules, which ramify throughout the cytoplasm (Fig. 15). The mitochondria are few in number and rounded or elongated in shape. The diameter of the rounded mitochondria ranges from 0.4 to 0.6 µm. The inner mitochondrial membrane forms numerous lamellar cristae (Fig. 16).

The nuclei of these cells are more or less rounded (the mean diameter is about 4 µm) or oval in

shape (the mean long axis is about 5 µm) and sometimes they appear indented. The nuclear envelope, which encloses. the nucleoplasm, consists as usual of two membranes. The chromatin is represerited by the euchromatin and heterochromatin. The euchromatin is represented by the electron-lucent nuclear material. On the other- hand, .the heterochromatin is represented by small electron-dense clumps on the inner aspect of the nuclear envelope and small dark patches scattered in the nucleoplasm. The nuclear envelope possesses nuclear pores, at areas where the heterochromatin is absent (Figs. 15 and 16).

The most prominent and dominant feature of the endocrine cells is the presence of secretory granules, which are numerous and are scattered in the cell cytoplasm. These secretory granules exhibit variable sizes and shapes in different cells. According to the dominant size of the secretory granules, the endocrine cells are differentiated into'three types: type-1, type-2 and type-3 cells.

Type-l of the endocrine cells

The type-l cells possess large, rounded secretory granules, which vary in diameter from 0.1 µm to 0.3µm (with a mean diameter of about 0.18 µ1m). These secretory granules are scattered in the cell cytoplasm and are usually aggregated at the basal region of the cell. These secretory granules consist of a homogeneous, electron-dense substance with variable densities. Each secretory granule is tightly surrounded by a membrane, but in some cases an electron lucent halo is interposed between the granule core and its surrounding or limiting. membrane (Fig. 17).

Type-2 of the endocrine cells

The type-2 cells possess small, rounded secretory granules, which vary in diameter from 0.05 µm to 0.1 µm (with a mean diameter of about 0.07 µm). These secretory granules are usually basally concentrated in the cell cytoplasm and, contain a homogeneous, electron-dense substance. Each secretory granule contains an electron-dense core which is sometimes separated from the surrounding membrane by an electron-translucent space, the halo (Fig; 18).

Type-3 of the endocrine cells.

The type-3 cells possess large secretory granules, which vary in diameter from 0.13 µm to 0.45µm (with a mean diameter of about 0.27 µm). These secretory granules are scattered throughout the cell cytoplasm and each granule consists of a small eccentric electron-dense core inside a rounded or irregular ni.embrane-limited vacuole. Some vacuoles appear empty without the electron opaque core (Fig. 19).

iii- The exocrine cells

The gastric glands are made up of a single type of cells, the exocrine cells, which are referred to as the oxynticopeptic cells. These cells secrete both the hydrochloric acid and gastric enzymes.

The oxynticopeptic cells, which possess electron-dense figures, are more or less cuboidal in shape (the mean length is about 11µm) and their luminal surfaces are either smooth or have numerous thin long microvilli (Figs. 20 and 21) The diameter of these microvilli is about 0.1 µm and their length varies from 0.5 to 2.9 µm. These cells possess a large number of spherical secretory zymogenous granules, which vary in diameter from 0.4 to 2µm. These granules are scattered throughout the cytoplasm and are aggregated at the apical surface of the cell among the numerous smooth tubules and vesicles. Each secretory granule contains an electron-dense core, which is tightly surrounded by a limiting membrane (Fig. 22).

The apical cell cytoplasm contains an extensive and extremely complex vesiculotubular system, which is composed of smooth membranous tubules (with a uniform diameter of 0.04 µm) and vesicles (with a mean diameter of about 0.1µm) (Fig. 22).

The rough endoplasmic reticulum is found intermingled with the mitochondria and consists of interconnected tubules and cisternae, which are studded with large numbers of ribosomes. On the other hand, the smooth endoplasmic reticulum consists of a few smooth tubules that lack the ribosomes. The free ribosomes are abundant and scattered throughout the cytoplasm (Fig. 22).

The mitochondria are large, numerous and are scattered throughout the cell cytoplasm. They are round, oval or elongated in shape with a diameter varying from 0.6 to 1.8µm (with a mean diameter of about 1.2 µm*).* The cristae are numerous and lamellar in shape (Fig. 22).

The nuclei are basally located in the cell and are usually rounded (with a mean diameter of about 4.5 µm) or oval (with a mean long axis of about 6 µm) in shape, and sometimes they appear indented (Figs. 20 and 21).

The lateral cell membranes form smooth contacts with the adjacent cells and lack interdigitations. The apical ends of the lateral cell membranes of the adjacent cells join together by the junctional complexes (Figs. 21 and 22).

B- The intestinal swelling of *Labeo niloticlIs*

The mucosa of the intestinal swelling of *Labeo ni/oticus* consists mainly of two types of cells: the columnar cells or enterocytes and goblet cells.

i- The enterocytes

The enterocytes, the most dominant cell type, are tall columnar cells (about 66µm in average

height), which rest on a prominent basement membrane. The apical cell membrane bears numerous regularly arranged cytoplasmic projections, the microvilli, which form the brush border (Fig. 23). These microvilli are covered by the glycocalyx and the upper parts of some of them are dense and form spherical bleb, called the capitulum (Krementz and Chapman, 1974) (Fig. 24). The mean length of the microvilli is about 0.77µm and the mean diameter is about 0.09µm. The apical cytoplasm, just below the microvilli, has no cellular organelles and forms the terminal web or net (Figs. 23 and 24). Invaginations of the apical cell membrane are frequently seen among the microvilli, forming the endocytotic channels (Fig. 24).

The enterocytes cytoplasm contains aggregated small lipid particles, the chylomicrons (Fig. 25).

The Golgi apparatus is well developed and is formed of one or two sets of parallel stacks, which are located near the nucleus in the laterobasal region of the cell (Fig. 26).

The endoplasmic reticulum is differentiated into two types, the rough and smooth endoplasmic reticula. The rough endoplasmic reticulum is well developed and consists of numerous long parallel tubules, which are mainly concentrated around the nucleus. On the other hand, the smooth endoplasmic reticulum is poorly developed and consists of a few short smooth tubules. The free ribosomes are scattered throughout the cell cytoplasm (Fig. 26).

The mitochondria are numerous, electron-dense, polymorphic (rounded, oval, elongated and ring-like in shape) and with variable sizes (the mean diameter is about 0.3 µm and the mean long axis is about 0.9 µm). The mitochondria are mainly aggregated at both the apical and basal regions of the cell (Figs. 23 and 24). The inner mitochondrial membrane forms numerous lamellar cristae (Fig. 24).

The cytoplasm is characterized by the presence of numerous lysosomes which are surrounded by distinct membranes (Fig. 25).

The enterocytes contain numerous and well developed microtubules, which are scattered in the cytoplasm and run parallel to the lateral cell membrane (Fig. 23). The mean diameter of these microtubules is about 0.02 µm (22 nm).

The nuclei of the enterocytes are large, oval in shape (with a long axis of about 10µm) and each one contains a prominent eccejjtric nucleolus (Fig. 23). The nuclear envelope is a double membrane consisting of the outer and inner nuclear membranes that encompass the nucleoplasm. The nuclear pores are found at intervals in the nuclear envelope. The euchromatin is represented by the pale granular areas of the nucleoplasm. On the other hand, the heterochromatin is represented by small dark granular patches scattered throughout the nucleoplasm and attached to the inner side of the inner nuclear membrane (Fig. 26).

The lateral walls of the cell membrane form smooth contact with the adjacent cells and lack interdigitations. The apical ends of these lateral walls of the adjacent cells join together by the junctional complexes (Figs. 23 and 24).

ii- The goblet cells

The goblet cells are flask-shaped and each cell is differenti"ated into two parts: a large swollen apical part and an elongated basal one (Fig. 27).

The swollen apical part is filled with large, oval or rounded homogeneous secretory granules. These secretory granules coalesce with each other, vary in both electron density and size (the mean diameter is about 1.2 µm) (Fig. 27). The cytoplasmic organelles are scarce, poorly developed and are aggregated in the elongated basal part. The Golgi apparatus is well developed, found in the supranuclear position and is formed of one or more sets of numerous parallel stacks (Fig. 28). The rough endoplasmic reticulum is found as scattered parallel tubules and cisternae, which are studded with the ribosomes. On the other hand, the smooth endoplasmic reticulum consists of a few parallel tubules that lack the ribosomes (Fig. 28). The mitochondria are oval in shape, small in size (the long axis is about 0.7 µm) and possess few lamellar cristae (Fig. 28). The nuclei of the goblet cells are oval in shape, (the long axis is about 4µm), basally located and each one possesses a prominent nucleolus and a well developed chromatin (Figs. 27 and 28).

4. Discussions

The wall of the stomach of *Schilbe mystus* and the intestinal swelling of *Labeo niloticus* consists mainly of four layers: the mucosa, submucosa, muscularis and serosa. This result conforms to that of Albrecht *et al.* (2001) in the omnivorous *Leporinus friderici* and *Leporinus taeniofasciatus,* Park and Kim (2001) in the carnivorous *Misgurnus* mizolepis, Diaz *et al.* (2003) in the carnivorous *Engraulis anchoita,* and Carrasson *et al.* (2006) in the carnivorous *Dentex dentex.*

The digestive system of the herbivorous *Labeo niloticus* lacks a true stomach and consequently lacks acid hydrolysis, so this fish may rely on the action of its strong pharyngeal mill and pharyngeal teeth to rupture the plant cell walls. These results agree with those of Klumpp and Nichols (1983) and Tibbetts (1997) in the herbivorous stomachless *Hyporhamphus melanochir* and *Arrhamphus sclerolepis krefftii,* respectively. Moreover, Logothetis *et al.* (2001) and Cinar and Senol (2006)

stated that the gut pH of the herbivorous stomachless *Atherinops affinis* and the omnivorous stomachless *Pseudophoxinus antalyae,* respectively, is around neutral or slightly basic. In addition, Logothetis *et al.* (2001) postulated that the pepsin activity is not present in the gut of *Atherinops affinis.*

The absence of a true stomach in the digestive system of the herbivorous *Labeo niloticus,* which belongs to the family Cyprinidae, may be related to phylogeny rather than to a character associated to the diet. This observation agrees with that of Weisel (! 962) who demonstrated that the carnivorous *Ptychocheilus oregonense,* which belongs to the family Cyprinidae, also lacks the true stomach. In addition, Reifel and Travill (1978) found that the digestive system of the cyprinid *Notemigonus crysoleucas* and *Pimephales promelas* also lacks the true stomach.

The digestive system of the carnivorous *Schilbe mystus* possesses a caecaltype stomach with a highly distensible caecum, which may be used as a reservoir. This observation agrees with that of Reifel and Travill (1978) in the piscivorous *Micropterus salmoides* and *Perca flavescens.* Moreover, Grau *et al.* (1992), in *Seriola dumerili,* and Carrasson *et al.* (2006), in *Dentex dentex,* stated that the carnivorous fishes with a predatory habit and irregular intake of large quantities of food possess a caecal-type stomach. The stomach of *Schilbe mystus* is morphologically divided into three regions: the cardiac, fundic and pyloric regions, but histologically it is divided into two regions only: the anterior wide sac-like cardio-fundic region and the posterior narrow tube-like pyloric one. These observations are in accordance with those of Al-Hussaini and Kholy (1953), in *Clarias lazera, Tilapia nilotica* and *Sargus vulgaris,* Grau *et al.* (1992), in *Seriola dumerili,* and Carrasson *et al.* (2006) in *Dentex dentex.* However, these observations contradict with those of Caceci *et al.* (1997) who showed that the stomach of *Oreochromis niloticus* is divided histologically into three regions: the initial, middle and terminal regions.

The gastric muscularis of the carnivorous *Schilbe mystus* is well-developed
and consists of unstriated muscle fibres arranged in two layers: an inner circular layer and an outer longitudinal one. The powerful gastric muscularis may act as a triturating device. These results agree with those of Grau *et al.* (1992), in the carnivorous *Seriola dumerili,* and Ostos Garrido *et al.* (1996), in the carnivorous *Anguilla anguilla.*

The cardio-fundic portion of the stomach of *Schilbe mystus* is characterized by the presence of gastric glands of the compound tubular type, while these glands are totally absent from the pyloric portion. These results agree with those of AI-Hussaini and Kholy (1953), in *Clarias lazera* and *Sargus vulgaris,* Cataldi *et al.* (1987), in *Sparus aurata,* Grau *et al.* (1992), in *Seriola dumerili,* Ostos Garrido *et al.* (1993), in *Oncorhynchus mykiss,* Satora (1998), in *Ancistrus multispinnis,* Albrecht *et al.* (2001), in *Leporinus friderici* and *Leporinus taeniofasciatus,* and Mai *et al.* (2005), in *Pseudosciaena crocea.* However, these results contradict with those of Osman and Caceci (1991), in *Tilapia niJotica,* Diaz *et al.* (2003), in *Engraulis anchoita,* and Carrasson *et al.* (2006), in *Dentex dentex,* who stated that the gastric glands are formed of the simple tubular type and are found in both the cardiac and pyloric portions.

The gastric mucosa of *Schilbe mystus* contains three different types of endocrine cells, which contain characteristic granules and show an extensive contact with the basement membrane. These cells may be involved in the secretion of various -kinds of hormones and play an important role in some physiological functions of the gut. These results agree with those of NoaillacDepeyre and Gas (1982), Podkowa and Goniakowska- Witalinska (2003), Lee *et al.* (2004) and Khidr (2006) who described three types of endocrine cells in the gastric mucosa of *Perca jluviatilis. Hypostomus plecostomus, Coreoperca herzi* and *Chrysichthys auratus,* respectively.

The gastric luminal cells of *Schilbe mystus,* which line the gastric lumen and gastric pits, are tall columnar cells with numerous apically concentrated granules. These secretory granules have a positive PAS reaction and may serve to protect the stomach epithelium from the auto-digestion processes caused by the gastric enzymes. These results confirm the findings of Gniu *et al.* (1992), in *Seriola dumerili,* Morrison and Wright (1999), in *Oreochromis niloticus,* Petrinec *et al.* (2005), in *Esox lucius* and *Silurus glanis,* and Carrasson *et al.* (2006), in *Dentex dentex.*

The secretory granules of the gastric luminal cells of *Schilbe mystus* are of variable shapes and sizes and are frequently seen in contact with the apical cell membranes to empty their contents into the gastric lumen via exocytosis. These results confirm what was shown by Ostos Garrido *et al.* (1993), in *Oncorhynchus mykiss* and Ostos Garrido *et al.* (1996), in *Anguilla anguilla.*

The lateral cell membranes of the gastric luminal cells of *Schilbe mystus* possess numerous complex interdigitations and the cohesion of these cells is ensured by the junctional complexes in the apical zones. These observations agree with those of Ostos Garrido *et al.* (1993), in *Oncorhynchus mykiss* and Ostos Garrido *et al.* (1996), in *Anguilla anguilla.*

The gastric gland bodies of the carnivorous *Schilbe mystus* are built up of a ingle exocrine cell

type, the oxynticopeptic cells, which probably perform the functions of the mammalian oxyntic and peptic cells related to the synthesis of both hydrochloric acid and pepsinogen, respectively. This result is in accordance with that of Grau *et al.* (1992), in the carnivorous *Seriola dumerili,* Ostos Garrido *et al.* (1996), in the carnivorous *Anguilla anguilla* and Petrinec *et al.* (2005), in the carnivorous *Esox lucius* and *Silurus glanis.* However, this result differs from that of Podkowa and Goniakowska-Witaliilska (2003) and Carrasson *et al.* (2006) who stated that the gastric gland bodies of *Hypostomus plecostomus* and *Dentex dentex,* respectively, are built up of two cell types: the light and dark cells.

In *Schilbe my!;tits* stomach, the oxynticopeptic cells posse~s combined features of both the oxyntic and peptic cells of the mammalian stomach. The apical cell membrane of the oxynticopeptic cell possesses numerous microvilli and the apical region of the cytoplasm contains a well-developed vesiculotubular system and numerous scattered mitochondria, which may be involved in the hydrochloric acid secretion. In addition, these cells possess a well-developed rough endoplasmic reticulum and numerous secretory granules, which may be involved in the pepsinogen secretion. These observations are in accordance with those of Ostos Garrido *et al.* (1993), in *Oncorhynchus mykiss,* and Ostos Garrido *et al.* (1996), in *Anguilla anguilla.* Moreover, Gallagher *et al.* (2001) stated that the vesiculotubular system of the gastric oxynticopeptic cells of *Logodon rhomboides* is typical of that found in the gastric oxyntic cells of the mammalian animals.

The luminal surfaces of the gland cells of *Schilbe mystus* are either smooth or have numerous thin and long microvilli. This observation agrees with that of Coetzee *et al.* (1991) who reported that the presence or absence of the microvilli of the gastric gland cells of *Hydrocynus forskahlii* may be ascribed to the secretion cyclic activity of these cells.

The muscularis mucosae is found in the pyloric portion of the stomach of *Schilbe mystus,* whereas it is absent from the cardio-fundic portion of the stomach. Reifel and Travill (1978) stated that the combination of a well developed musculature and the absence of the gastric glands of seven teleost species may indicate that the primary function of the pyloric region of the stomach is the mixing and pushing the food distally.

The enterocytes of the intestinal swelling of *Labeo nilotieus* contain a few numbers of endocytotic channels. Noaillac-Oepeyre and Gas (1974 and 1976), in *Cyprinus earpio* and *Tinea tinea,* respectively, and Olsen *et al.* (1999), in *Salvelinus alpinus,* suggested that the endocytosis of lipid is not a major route of lipid absorption in the anterior intestine enterocytes. Moreover, Stroband and Oebets (1978) showed that the anterior and middle segments of the intestine of the herbivorous *Ctenopharyngodon idella* possess endocytotic vesicles to uptake protein macromolecules.

The tips of some microvilli of the enterocytes of the intestinal swelling of *Labeo nilotieus* possess blebs or capitula, which may increase the apical surface and intensify penetration. Similar results were reported by Kuperman and Kuz'mina (1994) in *Esox lucius, Lota Iota* and *Abramis brama.*

The enterocytes of the intestinal swelling of *Labeo nilotieus* contain small chylomicron particles. This observation suggests that the enterocytes of the intestinal swelling are involved in lipid absorption. This suggestion is in accordance with that of Stroband and Oebets (1978), in *Ctenopharyngodon idella,* Anderson (1986), in *Gire/la trieuspidata,* Oeplano *et al.* (1989), in *Dicentrarehus labrax,* Olsen *et al.* (1999), in *Salvelinus alpinus,* Gallagher *et al.* (2001), in *Lagodon* rhomboids, Caballero *et al.* (2003), in *Sparusaurata,* Kozaric *et al.* (2004), in *Merluceius merlueeius,and* Carrasson *et al.* (2006), in *Dentex dentex.* Moreover, Fontagne *et al.* (1998), in *Cyprinus carpio* stated that the intestinal fat droplets can be interpreted as a temporary storage form of the reesterified fatty acids.

The enterocytes of the intestinal swelling of *Labeo nilotieus* contain a few numbers of endocytotic channels. Noaillac-Oepeyre and Gas (1974 and 1976), in *Cyprinus earpio* and *Tinea tinea,* respectively, and Olsen *et al.* (1999), in *Salvelinus alpinus,* suggested that the endocytosis of lipid is not a major route of lipid absorption in the anterior intestine enterocytes. Moreover, Stroband and Debets (1978) stated that the anterior and middle segments of the intestine of the herbivorous *Ctenopharyngodon idella* possess endocytotic vesicles to uptake protein macromolecules.

The mucosa of the stomach of *Sehilbe mystus* and the intestinal swelling of *Labeo nilotieus* contains numerous lymphocytes, which may play a role in protecting the fish from pathogenic organisms. This result agrees with that of Osman and Caceci (1991), in *Tilapia nilotiea* and Park and Kim (2001), in *Misgurnus m izolepis.* Moreover, Temkin and McMillan (1986) stated that the gut of *Carassius auratus* lacks the discrete aggregations of lymphoid tissue, such as the Peyer's patches of mammals, but possesses a diffused lymphoid tissue as numerous lymphocytes.

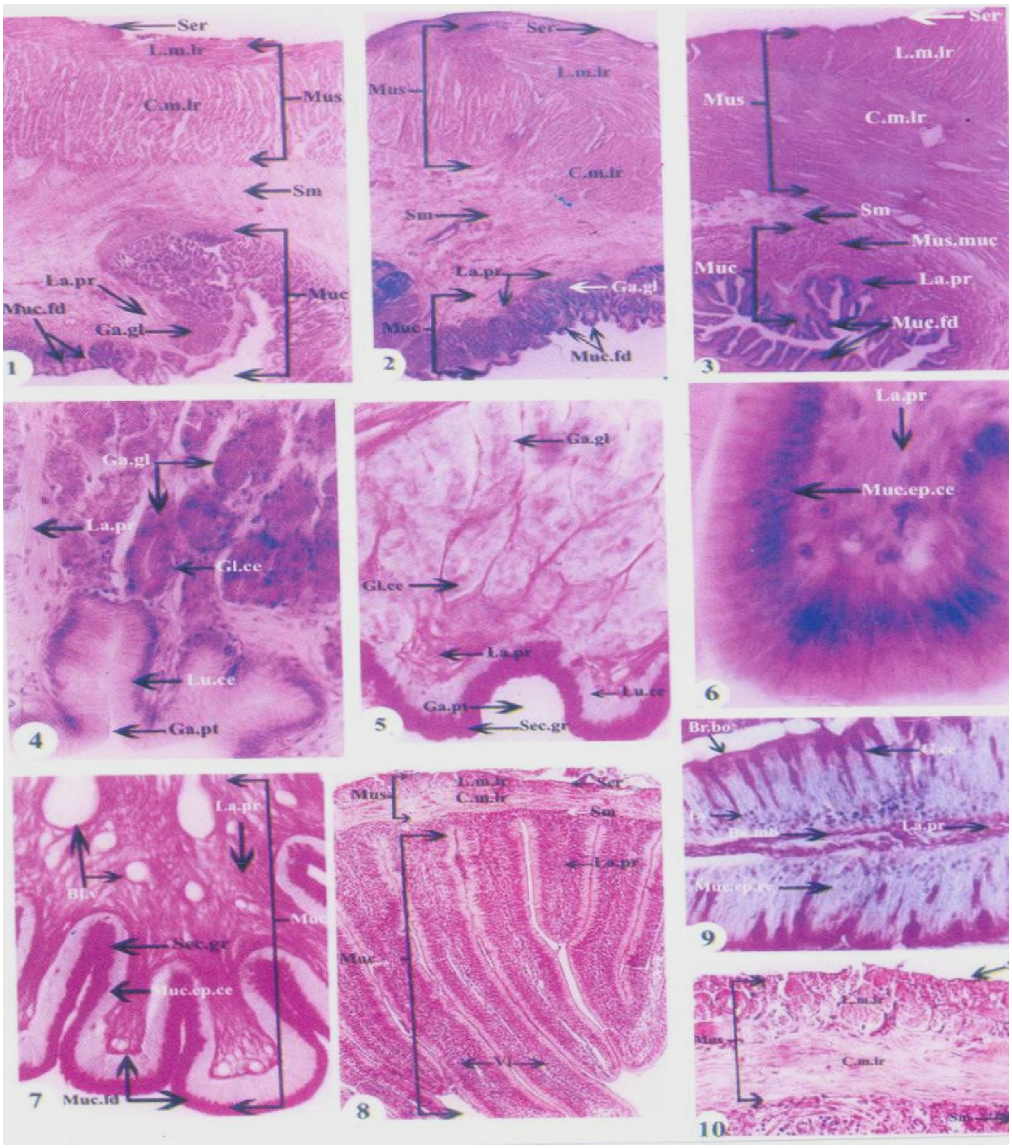

Figure (1): Photomicrograph of an L. S. of the cardiac portion of the stomach of *Schilbe mystus* showing the mucosa, submucosa, rriuscularis, serosa, mucosal folds, gastric glands, circular muscle layer, longitudinal muscle layer and lamina propria. H & E stain, X 38.

Figure (2): Photomicrograph of an L. S. of the fundic portion of the stomach of *Schilbe mystus* showing the mucosa, submucosa, muscularis, serosa, mucosal folds, gastric glands, circular muscle layer, longitudinal muscle layer and lamina propria. H & E stain, X 38.

Figure (3): Photomicrograph of a T. S. of the pyloric portion of the stomach of *Schilbe mystus* showing the mucosa, submucosa, muscularis, serosa and mucosal folds, muscularis mucosae, circular muscle layer, longitudinal muscle layer and lamina propria. H & E stain, X 38.

Figure (4): Photomicrograph of an L. S. of the cardlo-fundic portion of the stomach of *Schilbe mystus* showing the mucosa with luminal and glandular cells, gastric glands, gastric pits and lamina propria. H & E stain, X 380.

Figure(5):Photomicrograph of an L. S. of the cardio-fundic portion of the stomach of *Schilbe mystus* showing the lurninal cells with secretory granules, glandular cells, gastric glands, gastric pit and lamina propria. PAS & H stain, X 380.

Figifre (6): Enlarged portion of Fig. (3) Showing the mucosal epithelial cells and lamina propria. H & E stain, X 1096.

Figure(7):Photofflicrograph of a T. S. of the pyloric portion of the stomach of *Schilbe mystus* showing the mucosal epithelial cells withthe.secretory granules, mucosal folds, lamina propria and blood vessels. PAS & H stain, X 380.

Figure (8): Photomicrograph of a T. S. of the intestinal swelling of *Labeo niloticus* showing the mucosa, submucosa, muscularis (circular and longitudinal muscle layers), serosa, villi and lamina propria. H & E stain, X 96.

Figure (9): Photomicrograph of aT. S. of the intestinal swelling of *Labeo niloticus* showing the mucosal epithelial cells, goblet cells, lymphocytes, brush border, basement membrane and lam ma propria. PAS & H stain, X 412.

Figure (10): Enlarged portion of Fig. (8) Showing the submucosa, muscularis (circular and longitudinal muscle layers) and serosa. El & E stain, X 380.

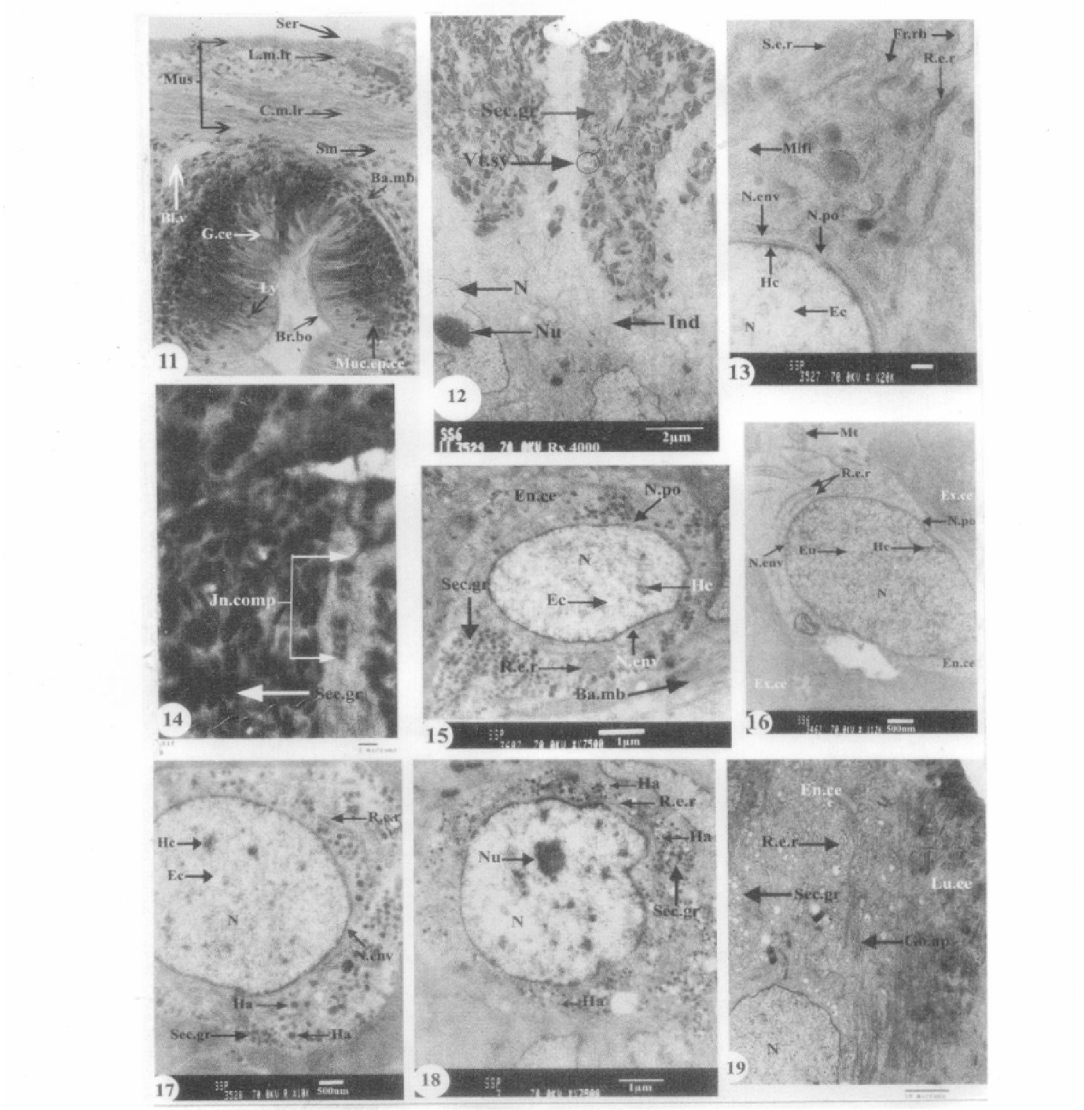

Figure (11): Photomicrograph of a T. S. of the intestinal swelling of *Labeo niloticus* showing the mucosa, submucosa, muscularis (circular and longitudinal muscle layers), serosa, ncosal epithelial cells, goblet cell, lymphocytes, brush border, basement membrane and blood vessel. H & E stain, X 375.

Figure (12): Electron micrograph of the gastric mucosa of *Schilbe mystus* showing the luminal cells with their secretory granules, vesiculotubular system. nucleus with a prominent nucleolus and lateral interdigitations.

Figure (13): Electron micrograph of a gastric luminal cell of Schilbe inystus showing the rough and smooth endoplasmic reticula, free ribosomes, microfilaments, nucleus, euchromatin, heterochromatin, nuclear envelope and a nuclear pore.

Figure (14): Electron micrograph of the gastric luminal cells of Schi/be mystus showing the secretory granules and junctional complexes.

Figure (15): Electron micrograph of an endocrine cell of Schilbe mystus showing the nucleus, nuclear envelope, nuclear pore, euchromatin, heterochromatin, secretory granules, rough endoplasmic reticul urn and basement membrane.

Figure (16): Electron micrograph of an endocrine cell, between two exocrine cells, of Schilbe mystus showing the nucleus, euchromatin, heterochromatin, nuclear envelope, nuclear pore, rnitochondria and rough endoplasmic reticulum.

Figure (17): Electron micrograph of type-i of the endocrine cells of Schilbe mystus showing the nucleus, euchromatin, heterochromatin, nuclear envelope, rough endoplasmic reticulum, secretory granules and halo.

Figure (18): Electron micrograph of type-2 of the endocrine cells of Schilbe mystus showing the nucleus, nucleolus, rough endoplasrnic reticulum, secretory granules and halo.

Figure (19): Electron micrograph of type-3 of the endocrine cells of Schilbe mystus showing the nucleus, rough endoplasmic reticulum, secretory granules Golgi apparatus.

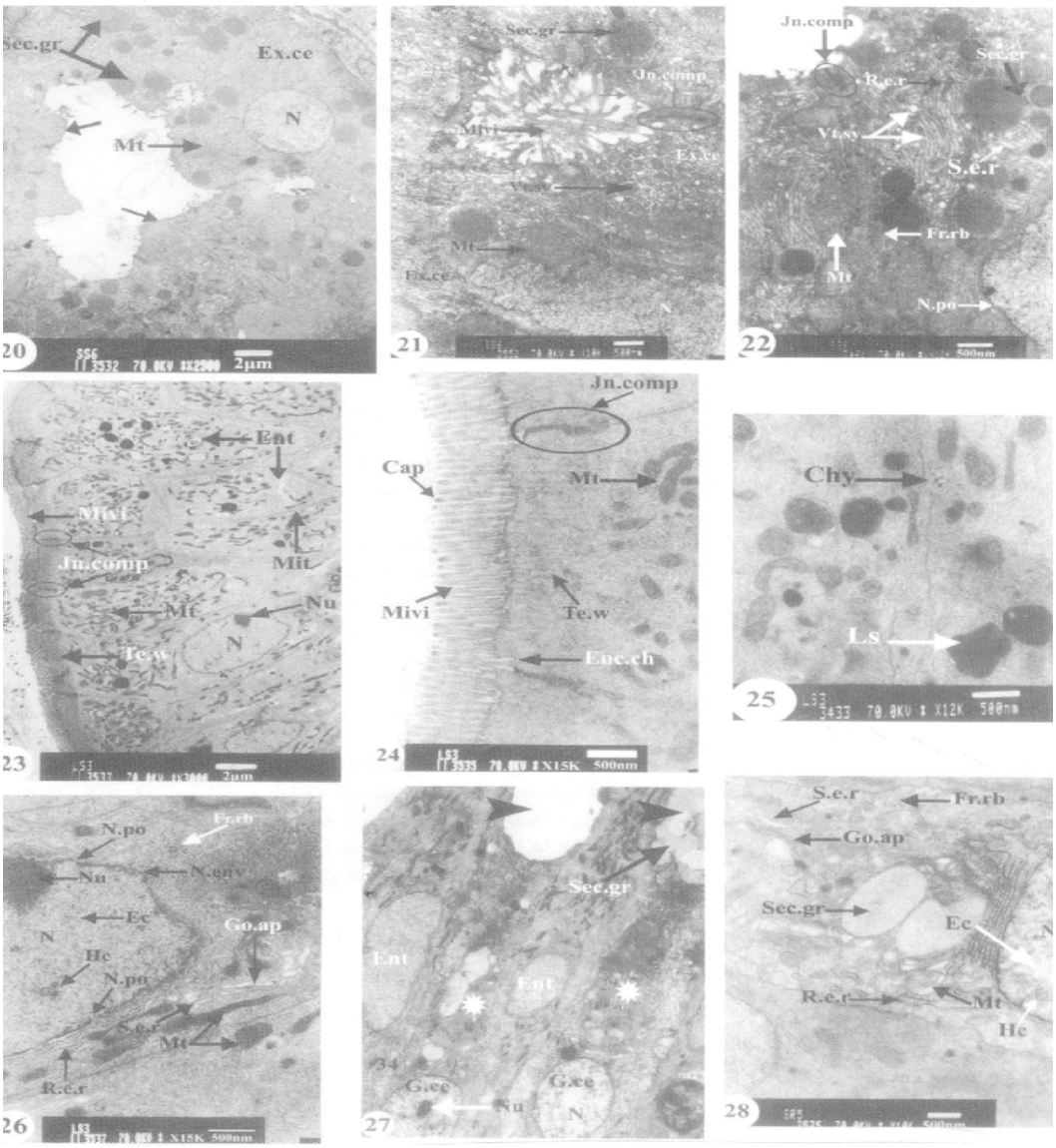

Figure (20): Electron micrograph of the gastric gland of Schilbe mystus showing the exocrine cells and their smooth apical cell membrane (arrows), nucleus, mitochondria and secretory granules.

Figure (21): Electron micrograph of the gastric gland of Schilbe mystus showing the exocrine cells with long microvilli on the apical cell membrane (arrows), vesiculotubular system, nucleus, mitochondria, secretory granules and junctional complex.

Figure (22): Electron micrograph of the exocrine cells of Schilbe mystus showing the vesiculotubular system, nucleus, nuclear pore, mitochondria, rough smooth endoplasmic reticula, Free ribosomes, secretory granules and junctional complex.

Figure (23): Electron micrograph of the intestinal swelling mucosa of Labeo niloticus showing the enterocytes, microvilli, terminal web, mitochondria, microtubules, nucleus, nucleolus and junctional complexes.

Figure (24): Electromicrograph of the apical part of the enterocytes of the intestinal swelling of Labeo niloticus showing the microvilli, terminal web, capitulum, endocytotic channel, junctional complex and mitochondria.

Figure (25): Electron micrograph of -the enteroc.ytes of the intestinal swelling of Labeo niloticus showing the lysosomes and chylomicrons inside the enterocyte.

Figure (26): Electron micrograph of the enterocytes of Labeo niloticus showing the Golgi apparatus, rough and smooth endoplasmic reticula, free ribosomes, mitochoñdria, nucleus, nucleolus, euchromatin, hêterochromatin, nuclear envelope and nuclear pores.

Figure (27): Electron micrograph of the intestinal swelling mucos of Labeo niloticus showing the enterocytes, goblet cells with the swollen apical parts (arrow heads) and elongated basal parts (asterisks), nucleus, nucleolus and secretory granules.

Figure (28): Electron micrograph of the elongaled basal part of the goblet cell of Labeo niloticus showing the secretory granules, Golgi apparatus, rough and smOoth endoplasmic reticula, free ribosomes, mitochondia, heterochromatin, euchromatin and nucleus

LIST OF ABBREVIATIONS

Ba.mb	Basement membrane	Ly	Lymphocyte
Bl.v	Blood vessel	Mit	Microuilaments
Br.bo	Brush border	Mit	Microtuhules
C.m.lr	Circular muscle layer	Mivi	Microvilli
Cap	Capitulum	Mt	Mitochondria
Chy	Chylomicrons	Muc	Mucosa
Fe	Euchromatin	Muc.ep.ce	Mucosal epithelial cells
En.ce	Endocrine cell	Muc.fd	Mucosal ftlcls
Enc.ch	Endocytotic channels	Mus	Muscularis
Ent	Enterocyte	Mus.muc	Muscularis mucosae
Ex.ce	Exocrine cell	N	Nucleus
Fr.rb	Free ribosomes	N.env	Nuclear envelope
G.ce	Goblet cell	N.po	Nuclear pore
Ga.gl	Gastric gland	Nu	Nucleolus
Ga.pt	Gastric pit	R.e.r	Rough endoplasmic
GLee	Glandular cell	reticulum	
Go.ap	Golgi apparatus	S.e.r	Smooth endoplasmic
Ha	Halo	reticulum	
He	Heterochromatin	Sec.gr	Secretory granules
md	Interdigitations	Ser	Serosa
Jn.comp	Junctional complex	Sm	Submucosa
L.m.lr	Longitudinal muscle layer	Te.w	Terminal web
La.pr	Lamina propria	Vi	Villi
Ls	Lysosomes	Vt.sy	Vesiculotubular system
Lu.ce	Luminal cell		

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Evaluation of Monosodium Glutamate Induced Neurotoxicity and Nephrotoxicity in Adult Male Albino Rats

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Abstract: Monosodium glutamate (MSG) is a food additive with a wide use in modern nutrition. The Current research concerned with studying the toxic effects of MSG on rats cerebrum and kidneys. Thirty six adult male albino rats were divided into three groups (each containing 12 rats); negative control group, positive control group (received 2 ml saline orally for 28 days) and MSG group (received 830 mg/ Kg. B. wt orally for the same period). Serum creatinine and blood urea nitrogen, urine analysis for urinary albumin excretion and histopathological examination for cerebrum, kidneys were examined in all groups. The results showed marked increase in the serum creatinine, BUN levels in MSG group as compared to control group. These changes were accompanied with a significant increase in the urinary excretion of albumin. These alterations were accompanied by variable histopathological changes of the examined cerebral and renal tissues. There were neurogenerative changes in the form of vacuolization, pyknosis, satellitosis and chroidal plexus congestion in the cerebral cortex. The renal tissue showed swelling in the lining endothelium of the glomeruli associated focal areas of glomerular atrophy. There was also hydropic degeneration of the tubules with tubular dilatation and hyaline casts. The inter-tubular spaces showed dilatation and congestion of the cortical blood vessels with focal hemorrhage between the tubules. Moreover MSG treatment induced up regulation of Bax protein in all examined tissues as compared to control rats indicating that MSG induced apoptosis. In conclusion, the results confirmed the neurotoxic and nephrotoxic effects of MSG, where Bax protein appeared to have a pivotal role in MSG induced apoptosis in these organs.
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Keywords: Monosodium Glutamate; Food Additive; Cerebrum; Kidneys; Apoptosis and Bax immunostaining

1. Introduction:

Monosodium glutamate (MSG) is a substance widely used as flavoring agent in the whole world. It is the sodium salts of glutamic acid. It is added to the food either as a purified monosodium salt or as a component of a mixture of amino acids and small peptides resulting from the acid or enzymatic hydrolysis of proteins **(Schwartz, 2004).** When it is added to food in relatively small quantities, the palatability of this food increases **(Vindini *et al.*, 2010).** There is a substantial evidence that the sensory basis for this effect is that MSG stimulates the sense of taste **(Garattini, 2000).**

Monosodium glutamate is absorbed very quickly from gastrointestinal tract and could spike blood plasma level of glutamate **(Schwartz, 2004).**

Glutamate is the most abundant amino acid in the central nervous system where it functions as an excitant neurotransmitter. It is especially highly concentrated in those regions of the brain that are essential in cognitive processes mediation; in the cerebral cortex, hippocampal gyrus dentatus and striatum **(Cekic *et al.*, 2005).**

Glutamate in high doses produce neuroendocrine abnormalities and neuronal degeneration **(Moreno *et al.*, 2005),** and oxidative damage in different organs **(Farmobi and Onyema, 2006; Pavlovic *et al.*, 2007).** On the other hand, glutamate in high concentrations, in particular in postnatal period, it acts as a neurotoxin (excitotoxin) **(Eweka *et al.*, 2011).**

The exact mechanism of neuronal cell death, induced by excitotoxins, still remains unknown. However, there is accumulating evidence suggesting that glutamate- induced toxicity can be mediated through necrosis and apoptosis **(Ankarcron *et al.*, 1998; Martin *et al.*, 2000).**

Kidneys are quite vulnerable to toxic injury because they are exposed directly to the blood plasma via open fenestrae of their glomerular capillaries. Moreover, it metabolized many toxic substances where the concentration of the toxin any be several hundreds times greater in the kidney than else **(Stine and Brown, 2006).**

Previous scientific investigations aimed at determining the effect of MSG on body organs **(Nwaopara *et al*., 2004; 2007a, b; 2008a, b).** There are some reports of the toxic effects of MSG on the pancreas **(Nwaopara *et al*., 2004),** liver (**Nwaopara *et al*., 2007b)** and kidney **(Nwaopara *et al*., 2008 a).**

Osfor *et al.* (1997) indicated that kidney, liver, brain, and heart weight were significantly increased in rats treated with MSG. **Diniz *et al.* (2004)** reported that MSG induced alterations in

metabolic rate of glucose utilization and decreased antioxidant defenses. Generation of reactive oxygen species in different body cells is known to induce damage to DNA, lipids and proteins, lipid peroxidation in cellular membrane due to damage of the polyunsaturated fatty acids in the cell membranes. These effects may lead to cellular death by apoptosis.

Researches in recent years have been focused on understanding the molecular targets for toxin induced apoptosis **(Stine and Brown, 2006).** The two main families of apoptotic regulators taken into consideration in mammals are the caspases**,** and the Bcl-2 family members, represented by Bcl-2, Bcl-x_L, Bcl-w, and Mcl-1 (cell death inhibitors) and by Bax, Bcl-x_s, Bad, Bak (cell death promoters) **(Kelekar and Thompson, 1998).**

Hence previous literature regarding the detailed histopathological changes in the brain and kidneys was insufficient especially regarding the exact molecular target for MSG induced cell death, so the aim of the present study is concerned with the possible biochemical, histological and Bax immunohistochemical changes that may occur in rats' cerebrum and kidneys following short term chronic toxicity of MSG.

Material and Methods

Material:

1- Chemical:

The chemical used monosodium glutamate (MSG) ($C_5H_9NO_4.Na$) from Al-Dawlya Chemicals Co., Egypt with Purity > 98% NT.

2- Kits:

- Biomerieux France kits (Boehringer, Germany) for estimation of blood urea nitrogen (BUN) and serum creatinine.
- ABC Diagnostic Kits (New Damietta, Egypt) for estimation of urinary microalbuminuria .

3- Experimental animals:

Thirty six adult male albino rats were used in the present study. The weights of rats ranged from 180- 200 grams and their ages ranged from 3-4 months and obtained from the Animal Farm, Faculty of Veterinary Medicine, Zagazig University, divided into 3 groups and caged under standardized environmental conditions. Passive preliminaries for seven days in order to adapt themselves to their new environment and to ascertain their physical wellbeing. They were housed in a separate well ventilated cages, under standard conditions, with free access to the standard diet and water ad libitum. The experiment was conducted at the animal house of Faculty of Medicine Zagazig University. The experiment was performed in accordance with the "Guide for the Care and Use of Laboratory Animals" (**Institute of Laboratory Animal Resources, 1996).**

Methods:

1- Experimental design:

For this study, thirty six (36) adult male albino rats were divided into three groups, each group consisted of twelve rats.

Group I (negative control group): kept with no injection till end of experiment.

Group II (positive control group): each animal of this group received orally 2 ml saline daily, parallel to the chemical treated group

Group III (monosodium glutamate (MSG) group): each rat received daily an oral dose of MSG equals 830 mg/kg b. wt. (1/20 of rat's oral LD50 which equals 16600 mg/kg b. wt.) **(Richard and Lewis, 1990)**.

This experiment lasted for 28 days. On the morning of the last day of the experiment, all rats were subjected for collection of 24 hrs of urine for assessment of urinary albumin excretion then they were weighted for recording the final total body weight and body weight gain percent. Then rats were anaesthetized by ether for collection of blood samples for assessment of kidney function tests then sacrificed and dissected for collection of cerebra and kidneys to be weighted for recording the relative cerebral and kidney weight and then to be subjected for histopathological examination.

2- Blood samples collection:

Retro-orbital blood samples were collected from all rats. The collected blood was incubated at 37ºC until clotted then centrifuged to separate the serum. The serum samples were maintained at (-20°C) to be used for measurement of kidney functions tests (blood urea nitrogen and serum creatinine).

3- Urine samples collection:

On the morning of the last day of the experiment, each rat was housed individually in a metabolic cage and was allowed to access water as these metabolic cages are supplied with drinking bottles. Funnels of suitable size were arranged at the bottom of the metabolic cages for collection of urine and perforated plastic discs were arranged in the funnels to retain fecal matter (**Abd-AlAziz *et al.*, 2008).** The 24 hrs urine sample was collected for each animal in a beaker arranged at the bottom of the funnel. The volumes of the 24 hrs urine samples collected in the beakers were noted individually and used for determination of urinary albumin excretion or microalbuminuria.

4- Assessment of kidney function tests (KFTs):

*** Blood urea nitrogen (mg/dL):** Biomerieux France kit Estimation of blood urea has been carried out using kit of biomerieux France. It was done as

described by **Kaplan (1965)** according to the pamphlet of Biomerieux France kit by enzymatic colorimetric method**.**

*** Serum creatinine (mg/dL):** Estimation of serum creatinine has been carried out using kit of Biomerieux France. It was done as described by **Bjurosson (1979)** according to the pamphlet of Biomerieux France kit by colorimetric method**.**

5- Assessment of urinary albumin excretion (µg/ml**):** Urinary albumin excretion or microalbuminuria (µg/ml) was determined by rapid colorimetric method using commercially available kits (ABC Diagnostics, New Damietta, Egypt) and according to manufacture instruction **(Schosinsky *et al.*, 1987)**. Microalbuminuria was expressed as µg/24 hours by multiplying the results by the urine volume (ml) in 24hours.

6- Histopathological Studies:

After scarification and dissection, cerebrum (frontal cortex) and kidneys were removed immediately and fixed in 10% formal saline**.** After fixation, kidneys and cerebra were embedded in paraffin blocks and processed for the preparation of 5 µ thickness sections. These sections were subjected for the following stains; Hematoxylin and Eosin (H& E) as described by **Wilson and Gamble (2002)** and Bax immunohistochemical staining as described by **Zhang *et al.* (2006).**

Immunohistochemical Methods:

Immunohistochemical staining for Bax was performed by using the Streptavidin/peroxidase method. After dewaxing, the slices were heated and boiled for 30 min in citrate buffer Solution (0.01M, pH6.0) for retrieval antigen. Each section was treated with 3% hydrogen peroxide for 20 min at room temperature to diminish nonspecific staining. After rinsing with PBS, the slices were blocked with 5% normal goat serum in PBS (0.1M, pH 7.4) for 30 min at room temperature. The slices were then incubated with the primary rabbit anti- Bax (1: 200 (Biotechnology, Santa Cruz, USA) overnight at 4 ° C. After rinsing with PBS, the specimens were incubated with biotinylated secondary antibody at room temperature for 30 min, and then re- incubated with peroxidase- labeled streptavidin for 15 min. The immunoreactivity was visualized by 3, 3′-diaminobenzidine tetrahydrochloride solution (DAB-Chromogen-Kit, Carpinteria, CA). The slides were counterstained with hematoxylin. Positive reactions appeared brown. Both cytoplasmic and nuclear staining of the cell was the only pattern of staining considered to be positive for Bax **(Zhang *et al.*, 2006).**

4- Statistical analysis:

Data were represented as means ± SD The differences were compared for statistical significance by student's t test. Differences was considered significant at $p < 0.05$. All statistical analysis were performed using Epi-Info version 6.1**.(Dean *et al.*, 2000).**

3. Results

1-Total and relative body weight changes:

All treated animals were survived to the end of experimental period. The data obtained from the mean body weights are given in Table (1). There were no statistical significance differences ($p>0.05$) as regard the mean values of the initial body weight(g), final body weight(g), percent of body weight gain (%), and relative body weights of cerebrum and kidney (mg/g) when comparing both control groups (groups I&II).

A statistical comparison of the mean values of the initial body weight (g) of MSG treated rats (group III) compared to those of the negative control group (group I) showed no statistical significant difference ($p>0.05$). While the mean values of final body weight (g) and the percent of body weight gain (%) of MSG group (group III) showed a statistically significant increase ($P<0.001$) when compared with those of the negative control group (Group I).

There was also a non significant difference ($p>0.05$) as comparing the mean values of the relative cerebral weight (mg/gm) in MSG group (group III) to those of negative control group (group I). In contrast, there was a significant increase ($p<0.001$) in the mean values of relative kidney weight (mg/ g) of MSG group compared to the negative control group.

2-Results of kidney function test (KFTs) and urinary albumin excretion:

Biochemical analysis of kidney function tests and microalbuminuria are shown in Table (2). There were no statistical significance differences ($p>0.05$) as regard the mean values of KFTs (BUN & serum creatinine) and urinary albumin excretion when comparing both control groups. Statistical comparison of mean values of KFTs and urinary albumin excretion between the **negative control group** (group I) and **MSG group** (group III) revealed significant increase ($p< 0.001$).

3- Results of histopathological examination of the cerebrum (frontal cortex):

The control groups (groups I &II) have the same histological and immunohistochemical findings. H&E stained cerebral sections of rats of control groups showed normal picture. Examination of H&E stained cerebral sections of the control groups showed the normal arrangement of the layers of the cerebral frontal cortex, the molecular layer

covered with pia matter, the granular layer, and the pyramidal layer (Figs. 1&2). On the other hand immunoperoxidase stained sections for Bax showed no immunoreactions for both nerve cells and endothelial cells (Fig. 7) .

Table (1): Effects of monosodium glutamate treatment on total body weights, relative cerebral weight (CW/BW) and relative kidney weight (KW/BW) compared to control groups.

Groups	Negative control group	Positive control group	MSG group
Initial Body Weight (g)	188.17 ± 7. 25	188.66± 6.87≠	189.25± 6.31≠.
Final Body Weight (g)	216.33± 8.44≠	217.67± 10.59≠	230.58± 3.55*
% of body weight gain	14.97%	15.83%≠	21.84%*
Relative cerebral weight CW/BW (mg/g)	1.67± 0.08	1.65± 0.07≠	1.59± 0.09≠
Relative kidney weight KW/BW(mg/g)	2.72 ±0.16	2.78 ±0.14≠	4.49 ±0.04*

Values are given as mean ±SD for group of 12 animals, CW/BW: cerebral weight compared to final body weight, KW/BW: kidney weight compared to final body weight . ≠ = non-significant difference compared to negative control; * = significant difference compared to negative control group.

Table (2): Effects of monosodium glutamate treatment on kidney function tests (BUN& S. creatinine) and urinary albumin excretion compared to control groups.

Groups	Negative control group	Positive control group	MSG group
Blood urea nitrogen (mg/dl)	**21.00±1.03**	**22.02± 1.00≠**	**38.5± 3.3***
Serum Creatinine (mg/dl)	**0.94±0.15**	**0.92± 0.14≠**	**2.18± 0.06***
Urinary albumin excretion (µg/24hrs urine)	**100 ±16**	**98 ±14≠**	**409 ±40***

Values are given as mean ±SD for group of 12 animals, ≠ = non-significant difference compared to negative control; * = significant difference compared to negative control group.

On the contrary, MSG treated rats (group III) presented signs of neurodegeneration with pyknosis and vacuolization. Examination of H&E stained cerebral frontal cortex sections of MSG treated rats showed most nerve cells distorted in shape and had deeply stained shrunken pyknotic nuclei. cells are surrounded by unstained areas . there was vacuolization of the neuropile surrounding the affected damaged nerve cells (Figs. 3&4). There was also focal aggregation of glial cells (gliaosis and satellitosis) (Fig. 5) and chroidal plexus congestion (Fig. 6). Immunoperoxidase stained sections for Bax showed positive immunoreactions in most of the nerve cells when compared to the control group (Fig. 8).

3- Results of histopathological examination of the kidneys:

The kidneys of rats' of both control groups (Groups I&II) showed normal histological picture of the kidney. The renal cortex is formed of Malpigian renal corpuscles, which are formed of tuft of capillaries (glomerulus), surrounded by Bowman's capsule. Renal space is preserved. Proximal and distal convoluted tubules were normal (Figs. 9&10).

Microscopic examination of the kidneys specimens of the rats of MSG group (Group III) after 28 days of treatment Showed variable pathological changes in glomeruli and renal convoluted tubules. Such changes exhibited an existence of hydropic degeneration of the tubular epithelium with vacuolization and tubular dilatation with intra-lumenal hyaline casts (Figs. 11&12). The presence of mononuclear inflammatory cellular infiltration in interstitial tissues were noticed (Fig. 12).

Also, they were swelling in the lining endothelium of the glomeruli associated focal areas of glomerular atrophy (Figs. 13&14). Dilatation and hyperemia in the intertubular cortical blood vessels and focal hemorrhage between the tubules were seen clearly (Fig. 14).

Immunolocalization of Bax in all examined kidney specimens of control groups revealed negative expression in both glomerular endothelium and renal tubular epithelium (Fig. 17).

Twenty eight days of treatment with monosodium glutamate resulted in positive immune reaction of Bax in glomerular endothelium and some renal tubular epithelial cells (Fig.18)

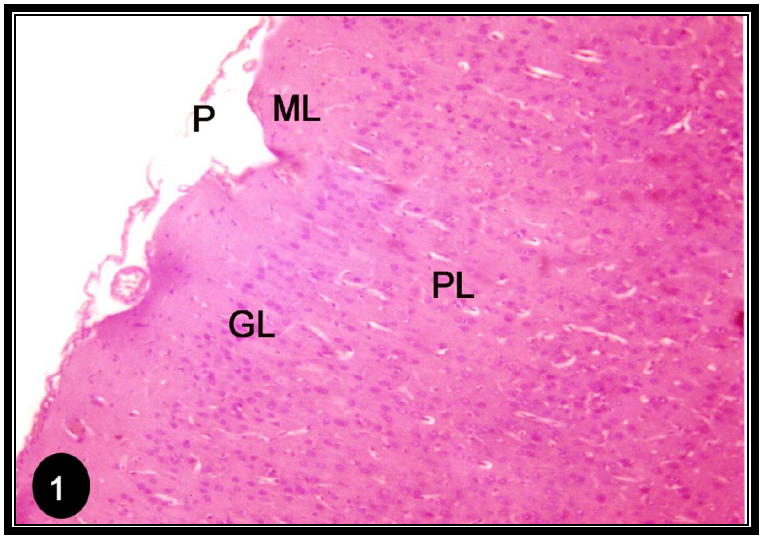

Figure (1): A Photomicrograph of a section in the cerebral cortex of a control rat showing the molecular layer (ML) covered with the pia matter (P), granular layer (GL), pyramidal layer (PL). H&E x 100.

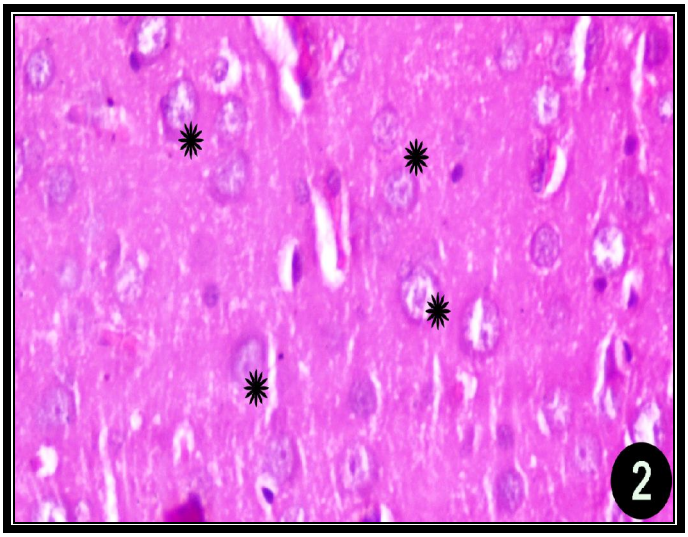

Figure (2): A Photomicrograph of a section in the cerebral cortex of a control rat showing the normal neurons of the granular layer with large vesicular nuclei (*). H&E x 400.

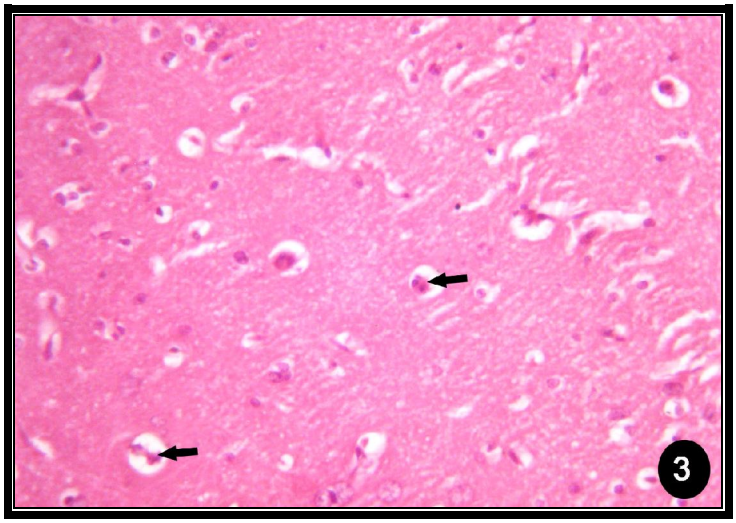

Figure (3): A Photomicrograph of a section in the cerebral cortex of MSG- treated rat showing most neuronal cells shrunken surrounded by spaces (Arrows).H&E x 200.

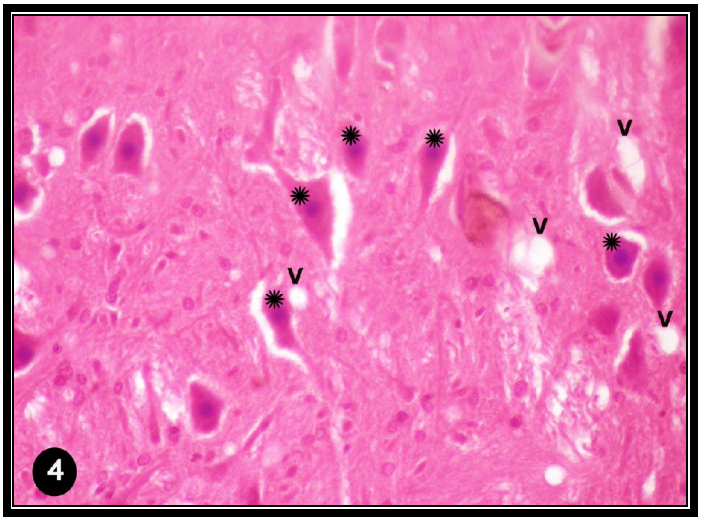

Figure (4): A Photomicrograph of a section in the cerebral cortex of MSG- treated rat showing most pyramidal cells appear distorted with deeply stained shrunken pyknotic nuclei, these cells are surrounded by unstained areas (*). There are vacuolizations (V) of the neuropile surrounding the affected nerve cells. H&E x 400.

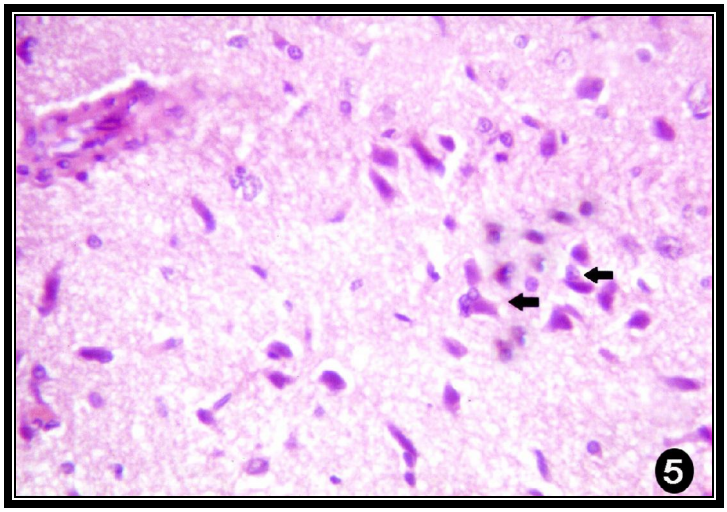

Figure (5): A Photomicrograph of a section in the cerebral cortex of MSG- treated rat showing focal aggregation of glial cells (gliaosis and satellitosis) (Arrows).H&E x 200.

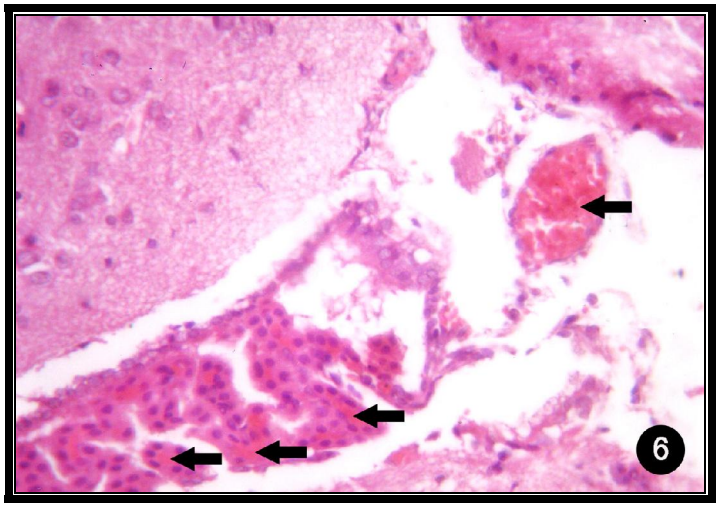

Figure (6): A Photomicrograph from a section in the cerebral cortex of MSG- treated rat showing chroidal plexus congestion (Arrow). H&E x 400

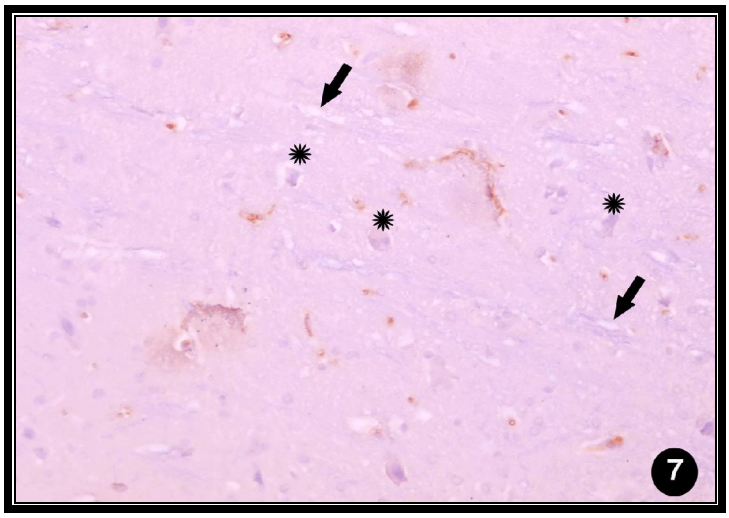

Figure (7): A Photomicrograph of a section in the cerebral cortex of a control rat showing negative immunoreactions for Bax protien in nerve cells (*) and endothelial cells (Arrow). immunoperoxidase for Bax x 400

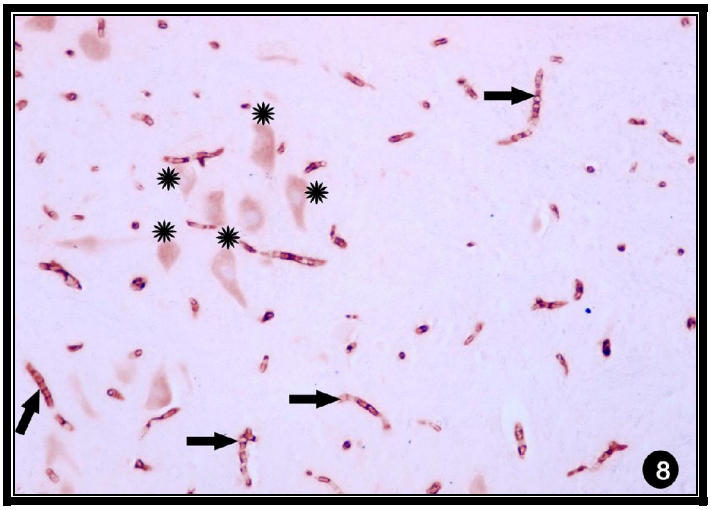

Figure (8): A Photomicrograph from a section in the cerebral cortex of MSG- treated rat showing positive immunoreactions for Bax protein in endothelial cells (Arrows) and weak reaction in nerve cells (*). immunoperoxidase for Bax x 400

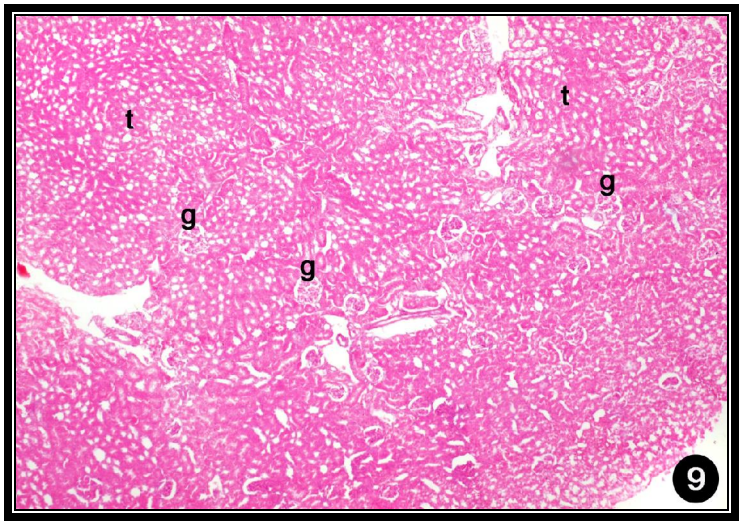

Figure (9): A photomicrograph of a section in a control rat's kidney showing normal glomerular tuft (g) and tubules (t). (H & E x40)

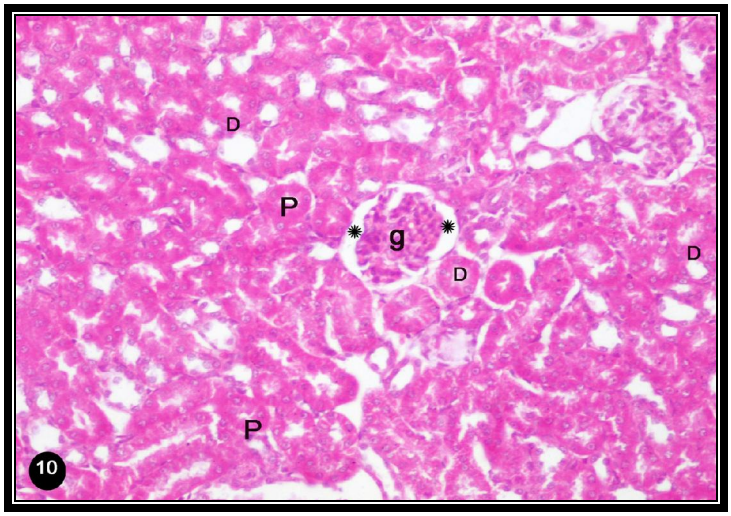

Figure (10): A photomicrograph of a section in the kidney cortex of a control rat showing renal corpuscle formed of glomerulus (g) surrounded with Bowman's space and preserved renal space (*). Note the proximal (P) and distal (D) convoluted tubules. (H & E x100)

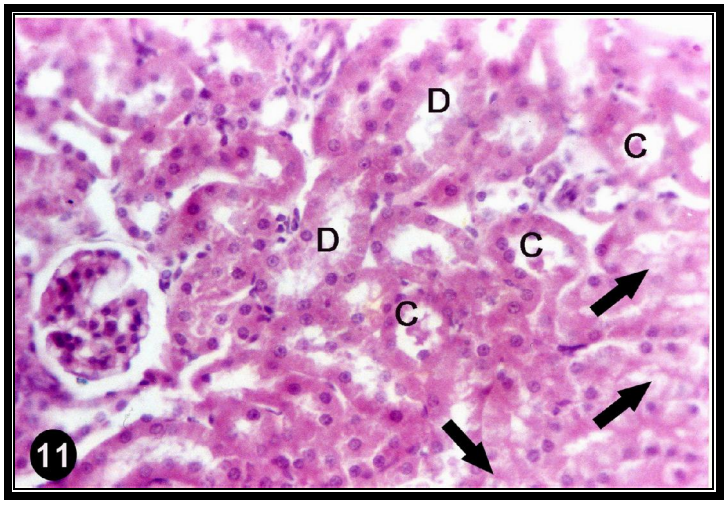

Figure (11): A photomicrograph of a section from the kidney of a rat of MSG group showing hydropic degeneration and vacuolization of the tubules (arrows), tubular dilatation (D) with hyaline casts (C) (H & E x200)

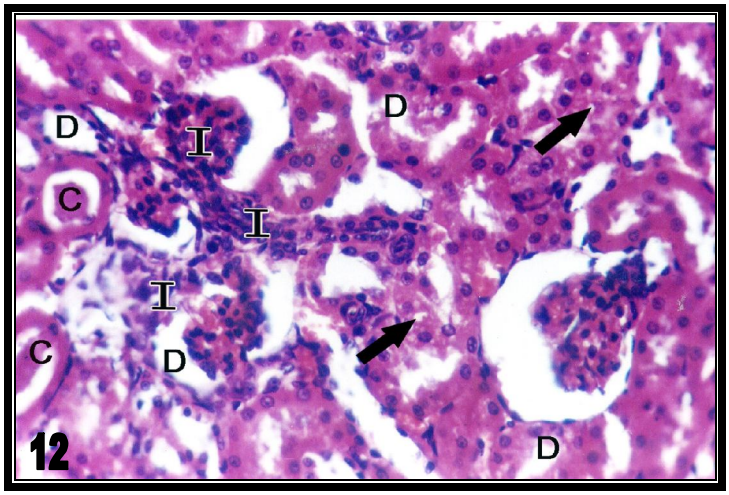

Figure (12): A photomicrograph of a section from the kidney of a rat of MSG group showing monocellular inflammatory cell infiltrates (I). Tubular dilatation (D), hyaline casts (C) and vacuolization of tubular cells are also observed (arrows) (H & E x200)

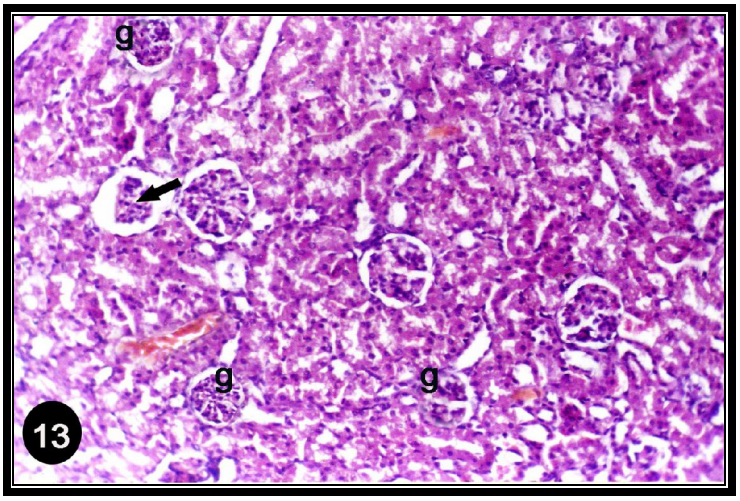

Figure (13): A photomicrograph of a section from the kidney of a rat from MSG group. Some glomeuli showing shrinkage (arrow) and the others showing swelling with partial loss of the Bowman's spaces (g). (H & E x100)

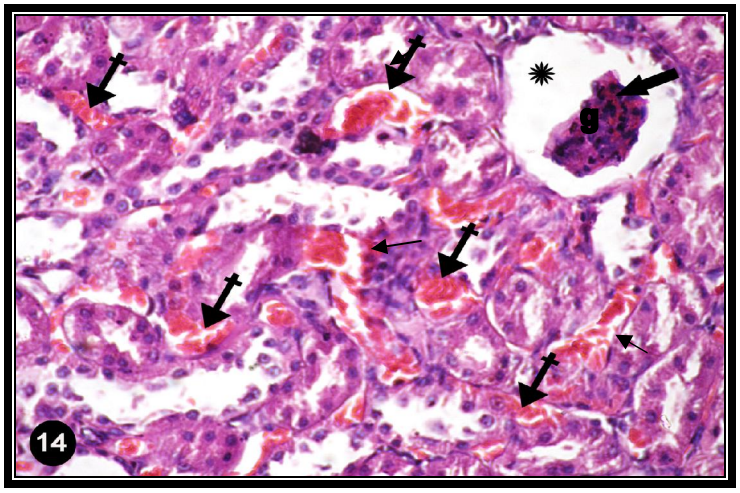

Figure (14): A photomicrograph of kidney section form a rat of MSG group showing severe shrinkage of the glomeulus (arrow) with increased in Bowman's spaces (*). There is also dilated congested interstitial blood vessels and interstitial hemorrhage in the inter tubular spaces (crossed arrows). (H & E x 400).

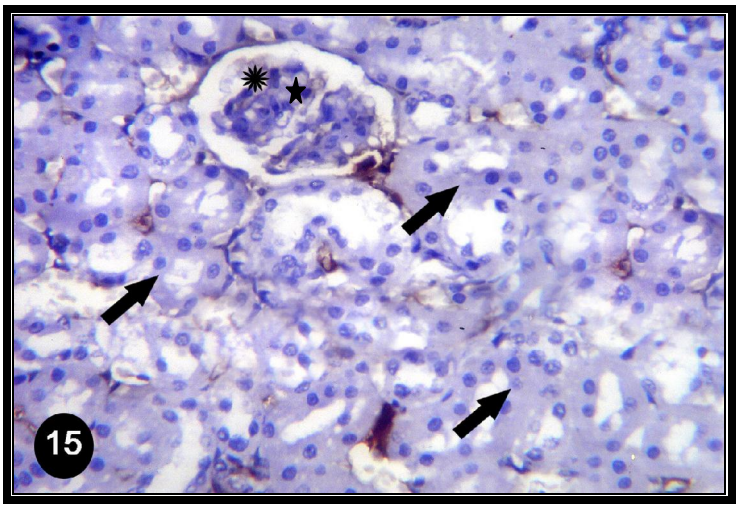

Figure (15): A Photomicrograph from a section in kidney of a control rat showing negative immunoreactions for Bax protien in glomeruli (*) and tubular cells (Arrow). immunoperoxidase for Bax x 400

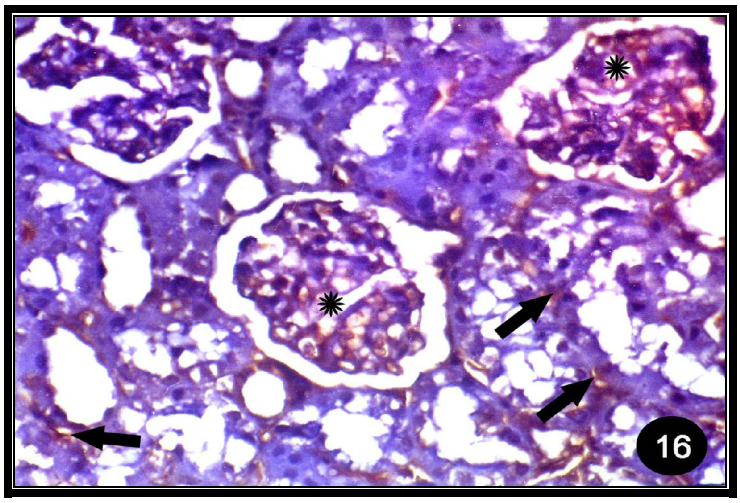

Figure (16): A Photomicrograph from a section in kidney of a rat of MSG group showing positive immunoreactions for Bax protein in mainly in glomeruli (*) and some tubular cells (Arrows). immunoperoxidase for Bax x 400

4. Discussion

Nowadays, monosodium glutamate (MSG) is frequently used as a flavor enhancer, the fact of which makes it one of the most applied food additives in the modern nutrition allover the world **(Garattini, 2000).**

In 1959, the U.S. Food and Drug Administration (FDA) classified monosodium glutamate as generally recognized as safe. Since that, FDA has sponsored extensive reviews on the safety of monosodium glutamate where most of the experts concluded its safety. They noted that doses as high as 2.1 g per kg for 30 days produced no adverse effects in males when given for a 70 kg male **(Beyreuther *et al.*, 2007).**

But accurate information on the daily intake of specific food additives by individuals is difficult to obtain especially for food additives that considered GRAS (generally recognized as safe) and so studying

their cumulative toxic effects should be a matter of concern **(Fennema, 1987).**

So the aim of the present study is concerned with the possible biochemical, histological and Bax immunohistochemical changes that may occur in rats' cerebra and kidneys following short term chronic toxicity for 28 days of MSG in a dose of 830 mg/kg that equals 1/20 of oral rat LD_{50}.

Results of the present study demonstrated several toxicological consequences in experimental animals secondary MSG treatment. These findings included a significant increase in the final total body weight, in the percent of total body weight gain and relative kidney weight.

These findings were consistent with the previous studies concerned with MSG induced obesity. It was found that monosodium glutamate has been shown to cause obesity in lab rats by down regulating hypothalamic appetite suppression and, thus, increasing the amount of food the lab rats consumed **(Hermanussen *et al.*, 2006).**

The negative effects monosodium glutamate were first observed in newborn mice by Lucas D R and Newhouse J P, 1957 who noted that monosodium glutamate induced elevation of glutamate level in the brain of mice that end in destruction of the neurons in the inner layers of the retina. Later, in 1969, John Olney discovered the phenomenon was not restricted to the retina, but occurred throughout the brain, and coined the term excitotoxicity **(Kandel *et al.*, 2000**).

In the present study monosodium glutamate treatment in a dose of 850 mg/kg has resulted in variable histopathological changes in the rats cerebra. These changes were in the form of neurodegenerative changes with pyknosis and vacuolization. Examination of H&E stained cerebral cortex sections of MSG treated rats showed most nerve cells distorted in shape with deeply stained shrunken pyknotic nuclei surrounded by unstained pale areas with vacuolization of the neuropile surrounding the damaged neurons. There was also chroidal plexus congestion and focal aggregation of glial cells (Gliaosis and satellitosis)**.**

Satellitosis observed here coincided with MSG induced neuronal cell death. As satellitosis means accumulation of neuroglial cells around neurons; seen whenever neurons are damaged **(Vijayan *et al.*, 1993).**

The results of the current study were in consensus with **Martin *et al.* (2000); Ortiz *et al.* (2006); and Pavlovic *et al.* (2007).** They reported treatment with glutamate as monosodium glutamate (MSG) induced severe neurochemical damage and neurotoxic effects on some brain regions.

Excessive accumulation of glutamate in the synaptic cleft has been associated with excitotoxicity **(Mallick, 2007).** Excitotoxicity is the pathological process by which nerve cells are damaged and killed by excessive stimulation by neurotransmitters such as glutamate and similar substances. This occurs when receptors for the excitatory neurotransmitter glutamate (glutamate receptors) such as the N-methyl- D- aspartate (NMDA) receptor are over activated.

Excitotoxins like NMDA and kainic acid which bind to these receptors, as well as pathologically high levels of glutamate, can cause excitotoxicity by allowing high levels of calcium ions (Ca2+) to enter the cell. Ca2+ influx into cells activates a number of enzymes, including phospholipases, endonucleases, and proteases enzymes. These enzymes go on to damage cell structures such as components of the cytoskeleton, membrane, and DNA **(Fujikawa, 2005).**

Moreover, the production of tumor necrosis factor- á (TNF- á), an inflammatory mediator, was significantly increased in rat's brain following glutamate treatment **(Chaparro- Huerta *et al.*, 2005).**

Additionally, it was also observed in the present study that MSG can induce a change in the pattern of expression of Bax protein in cerebral tissues.

These results are in agreement with **Goa *et al.* (2008).** They found that glutamate treatment for neuronal cells resulted in over expression of Bax protein and neuronal cell death with no effect on Bcl_2 expression. These observations could be attributed to glutamate ability to interact with NMDA receptors which induces mitochondrial calcium increase, free Radical generation, activation of proteases, phospholipases and endonucleases, and the transcriptional activity of apoptotic programmes **(Pelligrini- Giampetro *et al.*, 1997).**

The mitochondria are essential for life. Without them, a cell ceases to respire aerobically and quickly dies, a fact exploited by some apoptotic pathways. Apoptotic proteins that target mitochondria affect them in different ways. They may cause mitochondrial swelling through the formation of membrane pores, or they may increase the permeability of the mitochondrial membrane and cause apoptotic effectors to leak out. Mitochondrial proteins are released into the cytosol following an increase in permeability. They binds to inhibitor of apoptosis proteins (IPA) and deactivates them, preventing them from arresting the apoptotic process and therefore allowing apoptosis to proceed. IAP also normally suppresses the activity of a group of cysteine proteases called caspases, which carry out the degradation of the cell, therefore the actual degradation enzymes can be seen to be indirectly

regulated by mitochondrial permeability **(Fesik and Shi, 2001).**

Mitochondrial permeability is regulated by various proteins, such as those encoded by the mammalian Bcl-2 family of anti-apoptopic genes. *Bcl-2* proteins are able to promote or inhibit apoptosis by direct action on mitochondrial permeability where Bax and/or Bak increase mitochondrial permeability, while Bcl-2, Bcl-xL inhibit it **(Dejean *et al.*, 2006).**

The present study indicated that MSG induced marked biochemical and histopathological alterations in the kidney tissues of rats. There was affection for both the brain and the kidneys. kidney damage usually is associated with functional changes that may have been detrimental to the health status of the experimental animal. The greater the severity of the insults the more rapid the progression of neuronal injury. The principle holds true for toxicological insult to the brain and other organs **(Eweka, 2007).**

It may have been inferred from the present study that prolonged administration and higher doses of MSG resulted in increased toxic effect on the kidney that may play a pivotal role in cerebral damage observed here in the present study.

The observed renal damage was accompanied with impairment of the renal biochemical parameters as there was a significant elevations in the mean values of BUN, serum creatinine and urinary albumin excretion in MSG treated rats as compared to control. These findings indicating impairment of kidney function in rats treated with MSG.

In this context **Attia *et al.* (2008)** found that administration of MSG resulted in impairment of some renal biomarkers reflected by the significant increase in urea and decrease in albumin serum levels.

The significant increase in microalbuminuria measured by the end of the present study reflecting changes in glomerular filtration rate, glomerular impairment and failure of the kidney to retain the plasma albumin (**Abd-Alaziz *et al.*, 2008).**

It is generally believed that the increased urinary albumin excretion in most renal insults is mostly glomerular in origin. This may be due to increased intraglomerular pressure, loss of negatively charged in the basement membrane, and increased basement membrane pore size **(Marshall, 2004).**

Furthermore, the biochemical alterations induced by MSG treatment in the current study were augmented by the observed histopathological changes in the examined renal tissues of rats treated with MSG. These histopathological alterations were in the form of swelling of the lining epithelium of glomeruli, hydropic degeneration and vacuolization of the renal convoluted tubules, blood vessels dilatation and focal hemorrhage between the degenerative renal tubules. Similar Results have been reported by Mitsumari ***et al.*** **(1998) and Attia *et al.* (2008).**

Additionally, it was also observed in the present study that MSG can induce a change in the pattern of expression of Bax protein in both glomerular endothelial cells and some tubular epithelial cells.

The changes of Bax and Bcl-2 expression have been described in various experimental renal models; ischemic renal injury, diabetic nephropathy, and glomerulonephritis **(Yang *et al.*, 2001)**.

Glomerular swelling observed in the current study could be attributed to a decrease in O_2 levels which causes a drop in aerobic respiration. To maintain ATP levels, the cells must rely more on glycolysis. Glycolysis leads to lactic acid builds up, which causes the intracellular pH to drop. An acidic environment in the cell causes dysfunction of the Na+/ K+ ATPas and consequent cell swelling due to an influx of Na+ and H_2O. In the present investigation, many renal tubules of the rat kidneys showed marked degenerative lesions under the effect of MSG. This is justifiable since the renal tubules are particularly sensitive to toxic influences, in part because they have high oxygen consumption and vulnerable enzyme systems, and in part because they have complicated transport mechanisms that may be used for transport of toxins and maybe damaged by such toxins. Also the tubule come in contact with toxic chemicals during their excretion and elimination by the kidneys **(Tisher and Brenner, 1989).**

The circulating MSG was dissociated into sodium (Na) and L- glutamate. A part of the L-glutamate in the cell conjugates, in order to be eliminated, and another part is transformed into glutamine. When this occurs, the cells try to repair some of the damages by using enzymes that are present in the smooth endoplasmic reticulum but the cell is not able to completely remove the excess glutamine **(Singh *et al.*, 2003).** Probably for this reason, the convoluted tubules showed cloudy swelling.

When L- glutamate arrives in high concentrations through the renal artery, the kidney tries to excrete it. The renal corpuscle receives the L-glutamate through the afferent arteriole, it is absorbed, filtrated and across the membrane damaging the cell **(Attia *et al.*, 2008).**

Alterations in the levels of lipid peroxides and antioxidants such as reduce glutathione, catalase and superoxide dismutase were observed in different organs and systems of adult rat during MSG treatment (**Ahluwalia *et al.*, 1996 and Choudhary *et al.*, 1996).**

In addition, many glutamate receptors have been demonstrated outside the CNS **(Attia *et al.*, 2008).**

Moreover, NMDA receptors (one of glutamate receptors) have been found in extraneuronal tissues, including pancreatic β cells, the male lower urogenital tract, kidneys, lymphocytes, and megakaryocyte. There is scant evidence regarding its physiological function in extraneuronal tissues, especially in the kidneys. overstimulation of NMDA receptors can modulate glutamate postsynaptic neurotransmission by generating Ca^{2+} channel openings, and by overloading (**Nagata *et al.*, 1995)** and excessive reactive oxygen species generation (**Conn and Pin, 1997).**

Ischemia, followed by reperfusion, impairs kidneys and contributes to renal dysfunction (**Avshalumov and Rice**, **2002)**. Ischemia-reperfusion or hypoxia-reoxygenation injury also evokes burst amounts of reactive oxygen species and Ca^{2+} overload in damaged renal tubules, triggering the entry of these tubular cells into apoptotic and necrotic cell death, and subsequently, to renal dysfunction (**Deng *et al.*, 2002).**

In conclusion, short term administration of MSG to rats induced different toxic effects on both the cerebra and kidneys of these rats. These toxic effects manifested by widespread neurodegenerative changes in the rats's cerebral cortexes, biochemical elevations of BUN, serum creatinine, and urinary albumin excretion, and alterations in renal histopathological architectures. On the other hand, MSG treatment induced upregulation of Bax protein (apoptotic inducer) in these tissues. So, we can concluded that the results of the present study confirmed the neurotoxic and nephrotoxic effects of MSG, where Bax protein appeared to have a pivotal role in MSG induced apoptosis in these organs.

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Technology of earthquake resistant solar systems used in solar earthquake parks

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Abstract: Earthquake is among the natural destructive catastrophes whose incidence imposes serious social and economical damages to human societies. The only way to save people is to pay attention to safety issues, providing the required facilities and equipments prior to earthquake occurrence. The first step is hence smoothing the way for establishing and equipping parks and places supplying the essential needs of people wherein the natural, permanent, and reusable energy is used for the purpose of providing light, air conditioning, cooking, and telecommunication requirements. Regarding such an approach, carrying out the project of solar park for earthquake in all regions of Iran with high probability of earthquake occurrence is of critical importance. The next step will be to use the technology of anti-earthquake solar systems in these parks, which paves the way for reaching the first objective. In order to supply the required energy for light, air conditioning, and also for preparing hot water for bath and washing in these earthquake solar parks, it is needed to install photovoltaic (PV) panels with essential equipments on floor or under the ceiling of the buildings which have been recognized to be resistant against earthquake in critical times. Accordingly, making use of the technology of solar systems and designing and making the buildings with maximum use from solar energy are of great significance.
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Keywords: Solar Park; Reproducible energies; Earthquake; Natural catastrophes; solar systems; Photovoltaic

1. Introduction

According to equilibrium theory, after earthquake occurrence in a country, the critical condition is initiated due to the generated imbalance in the society. Why do we always observe the loss of thousands of our compatriots and then managing these conditions and eventually be proud by presenting the statistics relating to rescuing some people, burying the died ones, and reconstruction of damaged regions? So, the real crisis management should be started now [1].

The anti-earthquake solar systems technology should be used in earthquake solar parks, cities, locations susceptible to earthquake, rural regions, large parks, and open sport places. Inspiring from the technology of mentioned systems, it is possible to make anti-earthquake solar packages from steel. Since the electricity is off by earthquake occurrence and makes trouble for agitated people, it is crucial to consider the batteries saving the solar or reusable energy in these packages for light use in environment, charging of the cell phones, communication and telecommunication systems, radio and television, and chillers in garners and places wherein drugs and vaccines are kept [2]. Concerning the mentioned information, geographical location of our country, the possibility of using solar energy in all seasons, and high occurrence probability of earthquake in most of Iran's cities and rural regions, making use of such packages is of considerable importance.

2. General characteristics of the scheme

Making use of the anti-earthquake solar systems technology in earthquake solar parks is a scheme which was suggested in 2008 by me (constant member of Iranian solar energy society) for the first time in Iran, which was then patented in the bureau for discovery patent and the main bureau for company registration and industrial ownership, after confirmation of respective organizations. It is noteworthy that by applying this scheme, mass production of anti-earthquake solar packages in all earthquake-susceptible regions of Iran will be possible and it can save people up to several days by the installed equipments in the case of earthquake. Technical characteristics of this anti-earthquake solar package keep it approximately 80% intact even if the buildings are collapsed through the earthquake. Also, it should be mentioned about the recyclability and effective life of this packages that, the average operational life of these solar modules is 25 years and many of components and raw materials of these modules are reusable and recyclable. As was mentioned above, the portion of energy release reaching the earth, which is caused by sun's mass decrease, is 35,000 times the energy consumption of earth habitants and there would be no need to consume any fuel if we can absorb one unit of these 35,000 units of energy. Thus, in order to supply the required energy for light, air conditioning, and also for preparing hot water for bath and washing in these

earthquake solar parks, it is required to install these earthquake-resistant solar packages with essential equipments on floor or in buildings which have been recognized to be resistant against earthquake in critical times. In general, the advantages of the scheme of "using anti-earthquake solar systems in earthquake solar parks", which has gained the approval of respective organizations (including the Iranian national center of strengthening, the bureau for discovery patent and the main bureau for company registration and industrial ownership, ministry of industry and mine, and a faculty member of an Iranian university) are as following:

- preventing the sudden collapse of the buildings and providing the time needed for escape
- optimal use of energy resources
- saving the transportation costs
- saving people's lives and health
- making mental security for habitants of the earthquake-susceptible region
- low cost of production process
- transportation possibility in least time
- being resistant against the earthquake
- offering the possibility to be used in cities and earthquake-susceptible regions, rural regions, large parks and open sport places, and the buildings which have been recognized as resistant against earthquake and in critical times
- possibility of mobile installation and being used in critical conditions
- Higher safety and no preventing factor regarding air and environmental pollution is one of the characteristics of these systems. For instance, it is possible to cook the required food for 20 persons in one hour by each of solar ovens without the danger of firing or environmental problems.
- possibility of centralized control of the complex
- ease of installation, control, and operation
- no need to daily, weekly, and monthly services
- frugality in required space up to 70 percent

Meanwhile, the target places for this scheme are as following:

- cities and earthquake-susceptible regions
- rural regions
- large parks and open sport places
- the buildings which have been recognized as resistant against earthquake and in critical times

Damages due to earthquake are specific and experienced ones which have been witnessed in past in Iran and throughout the world. Building relatively high buildings exactly on fault line and also the expansion of towns in earthquake-susceptible cities indicates a programmed progress of such towns and the hypothesis is rejected that this expansion is carried out by people neglecting the rules of urban development. So, the responsible person is the one who permits building in these regions [3]. Fortunately, man in present world has come to the conclusion that, it is essential to transform reusable energies to other usable energy types via the technology of solar systems, which belongs to present era. Regarding this, a suitable method for using reusable resources is the application of photovoltaic systems. These systems are based upon direct transformation of solar energy into electrical energy, which will be discussed later.

Table 1- Properties of PU-5KW/48 and PU-15KW/48 system

Number	Description	Characteristics	Number in PU-5KW/48 system	Number in PU-15KW/48 system
1	Solar electricity panel	45 W, 2.76 A	112	352
2	Controller charger	48 W, 30 A	7	7
3	Battery	600 Ah	120	400
4	Holder system	Metallic/installed on floor	7	22
5	Inverter	48.220 KW, A	1	2
6	Wiring of the system	----------	----------	----------
7	Electronic equipments and control panels	----------	----------	----------

Table 2- capability of photovoltaic systems in some regions of Iran

Region	Sun's emission KWh/m^2/day	Regions	Application
1	5.2 – 5.4	South parts of Iran and some parts of the central regions (Kerman and Fars)	----------
2	4.8 – 5.2	Central regions and coastal parts near Persian Gulf and Oman Sea (Tehran, Semnan, Isfahan, Khorasan, Yazd, Sistan and Baluchestan, Bandar Abbas)	Hot water generation systems, heat and cold generation systems, systems for desiccating agricultural products, production of distilled water, freshwater Cannes, and solar powerhouses
3	3.8 – 4.5	Mountainous regions (East and West Azarbaijan)	Solar heat system (in summer, water produced with low temperature)
4	2.8 – 3.8	Coastal regions of the Caspian Sea (Gilan and Mazandaran)	Solar heat system

3. Development steps of photovoltaic systems

Photovoltaic systems are energizer systems which generate electricity from solar light without making use of moving mechanisms or chemical ones. In other words, these systems produce clean and reliable energy without consuming fossil fuels. Research relating to photovoltaic technology has been initiated from a hundred years ago. In 1873, the British scientist Willogbi Smith found out that selenium is sensitive to light. He resulted from his experiments that Selenium's ability in conducting the electricity has a direct relationship with the light emitted to it. In 1880, Charles Fritz accomplished in manufacturing the first electrical solar cell. This product could generate electricity without consuming raw materials and created no heat or noise. The research was however inert until 1905, when Albert Einstein presented his theory on photovoltaic effect. His theory caused a revolution in electricity generation; nevertheless little progress was made in the field due to high costs and low efficiency in production. In the early 1950s, during their studies on systems of remote communication and discovery of novel sources of energy, researchers at Bell laboratories found out the sensitivity of Silicon, the second abundant element on earth, to sun's light. They realized that when this element is used with a specific level of impurity, it will generate energy with considerable voltage. These researches led to production of the first silicon solar cell in 1954 with 60% of efficiency. Afterward, this technology was first utilized in rural telecommunication station in Georgia State. In early 1960s, the scientist in NASA installed a system of 108 solar cells on Vanguard satellite in order to generate abundant, light, reliable, and suitable energy out of earth's atmosphere. Till then, photovoltaic systems have been applied on most satellites and aerospaces. Nowadays, more than 200,000 houses in America use such a technology and these systems are utilized worldwide in a wide range. This method of energy generation is used in different kinds of communication, irrigation, filtering water, providing light, space and sea navigation, etc [4].

The process of electricity generation in photovoltaic system is simpler and less harmful to environment, compared to other common energy resources. In this process, the light particles which are called photons penetrate the cells and generate electrical current via release of electrons from silicon atoms. Electricity is generated until the light is emitted into the cell. These cells do not consume electrons like the batteries, since they are transformers which transform solar energy into electricity. It should be mentioned that electricity generation by modules is completely without danger [5].

4. Durability of photovoltaic components

Operational life of solar modules was considered in previous years to be 10 years on average, but it has reached 25 years due to technical advances. Meanwhile, many of the components and raw materials of these modules are reusable and recyclable. For instance, the glasses, plastic boxes, and metallic hanks are reusable; however some parts such as semiconductors cannot be recycled [6].

5. Components of photovoltaic systems

a) Photovoltaic cells

These cells are thin squares, discs, or semiconductor films which generate sufficient voltage and current when they are under the emission of sun's light.

b) Module and panel

Module is a set of photovoltaic cells which are placed layer by layer in a glass container. A set of several modules is called a panel.

c) Array and charger controller

Several panels connected to each other through wiring with specific voltage are called an array. Charger controllers are equipments which regulate and control the voltage of batteries and prevent from probable damages to batteries.

d) Battery storage (battery bank)

It is a device which stores the produced DC electrical energy in itself.

e) Transformer

It is a device which transforms DC current to AC current for use.

f) DC loads

These are tools, motors, and equipments which use DC current.

g) AC loads

These are tools, motors, and equipments which use AC current.

6. Types of photovoltaic systems

Photovoltaic systems have different types. The simplest form of them provides the required energy of watches and calculators. More complex systems provide the needed electricity of houses, factories, and other places by connecting to consumption network. Generally, these systems are utilized in different forms. For example, some home systems are equipped with energy storage batteries for use in night. Some systems use DC equipments and there is no need to transformers. Some other systems make use of transformers and use both AC and DC currents. Different types of photovoltaic transformers are mentioned in following.

Solar lamps and photovoltaic chargers used in radio batteries are of this kind and have a good market. In this system, all parts are integrated and rechargeable batteries are utilized instead of normal batteries.

The simplest and cheapest photovoltaic systems are designed for daily use. An example of these systems is illustrated in Fig. 2. These systems usually include modules which have no storage components and generate electricity by direct sun emission. Instances of this type are some fans, blowers, propellers for heat energy distribution in water heating systems, and the devices which use solar energy, like calculators and watches.

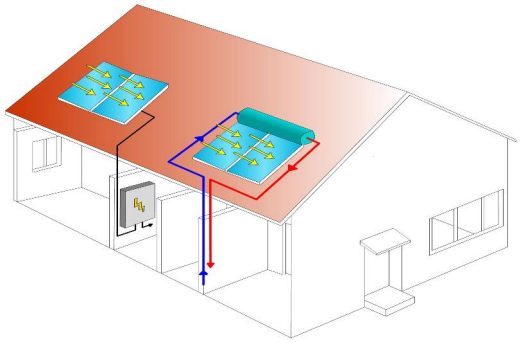

Fig 1- Thermal and electrical systems in the solar house

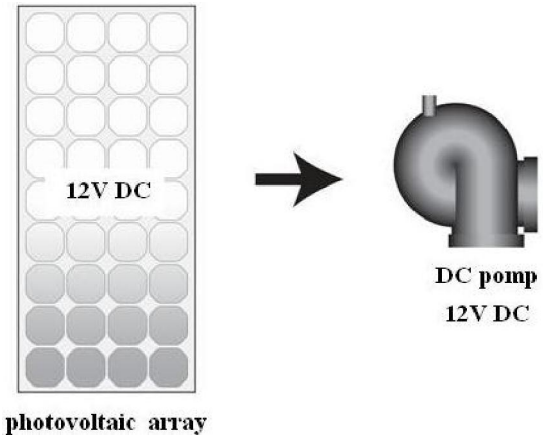

Fig 2- Daily-use systems

In order to use photovoltaic systems in night or cloudy air, the systems with storage batteries are used. Fig. 3 shows an example of such a system. These systems may include small devices, such as flashlight with one battery, and large machinery with numerous industrial batteries. The significant point about rechargeable batteries is that they should be charged after being completely discharged for more durability. Size and shape of battery source should be designed consistent with operation o system's voltage, amount of use in night, and weather condition, etc. In some types of these systems, a charge controller has also been designed which prevents the batteries from being over-charged or being abnormally discharged when the module is disconnected from battery source. This is effective in quality maintenance and durability of the battery.

Photovoltaic modules generate DC electrical energy by sun's emission; however most electrical devices need AC energy. Therefore, the photovoltaic systems must have a transformer for converting DC energy to AC. These transformers increase the flexibility of the system and offer facilities, but they results in cost increase.

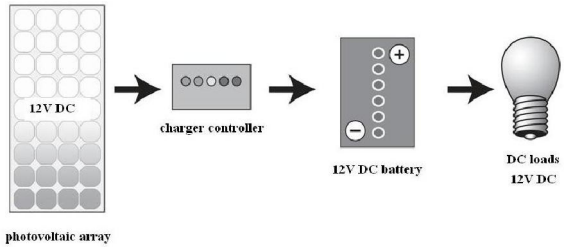

Fig 3- DC system with storage battery

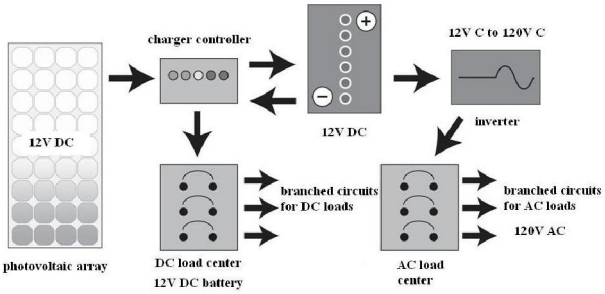

Fig 4- A system with DC and AC loads

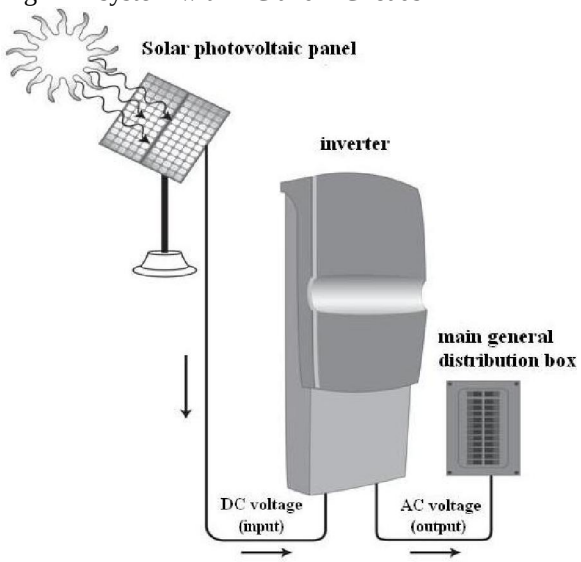

Fig 5- Systems connected to urban electricity network

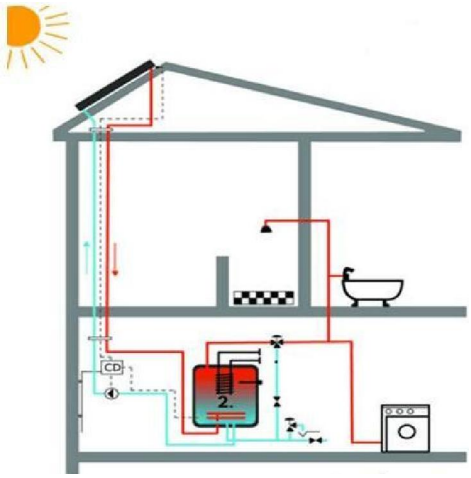

Fig 6- The system providing hot water for consumption in the solar package

These systems require no storage battery since the electricity network stores the energy itself. The system owner sells the excess generated energy to urban network and receives from this network in case of need. Subsequently, the conditions should be prepared such that energy exchange between the owner and urban network is possible. For this reason, some companies of urban electricity network give counters to their customers which determine the amount of electricity exchange [6].

Most people do not gain all of their required electrical energy only from photovoltaic system and they use some other types of energy. In hybrid systems, a diesel or gas motor generates energy which can be effective in reducing the initial costs. Preparations should be made in photovoltaic systems for unsuitable weather conditions. For this purpose, the batteries used should be large enough and be capable of providing the essential energy when needed. An advantage of hybrid systems is that they have at least two independent charging systems. As an example of these systems, the photovoltaic modules with wind turbines have mentioned property and possess the third battery charging resource, other than the advantages of photovoltaic and generating systems.

7. Solar system of water heater

Solar water heater with polymer collector has high efficiency, can be easily installed by low investment cost, and can provide up to 330 days hot water for consumption. In general, the required hot water can be obtained by receiving the sun's heat by flat solar collectors. In a solar water heater, water is heated in a flat thin black vessel. The heated water circulates in spiral pipes by a thermosiphon. These pipes are located spirally in the tank where water heats there; so thermal exchange takes place.

The solar water heater is utilized for heating the water which circulates through its pipes. In direct heating system, water is heated during its pass through flat and glass plates (solar collectors) which are located on the ramp roof toward south by slope angle of 15 to 50 degrees, whose position is less than 45 degrees toward southeast or southwest. The heated water is then stored in a tank above the collector. In most solar water heaters, there is also an auxiliary heating system which increases the water temperature in days that solar energy is not enough for providing heat to obtain hot water. Natural gas, liquid gas, or solid fuel is used in the source of the auxiliary system. Common solar water heaters in world market are closed-cycle system (main pressure), thermosiphon system with separate tank (low constant pressure), and compulsory circulating system (pumping by the main pressure) [7].

The solar water heater system appended to the solar package includes a set of two-layer source, collector, and the connecting pipes. In some types, a pump and a simple thermostat is utilized. Solar emissions are absorbed via the collector and transfer heat to the liquid in connecting pipe, which is then stored there in a source. The collector is usually installed on the mentioned package with a suitable angle and the optimum value of collector's deviation angle for heating is the geographical latitude plus 15 degrees toward south. Furthermore, computer predictions about collectors show that each 10m2 collectors can yearly collect 10,000 kW to 12,000 kW of energy, which is beyond the total heat needed for mentioned packages.

Auxiliary heater (electrical or gas); storage tank; hot water to house; input cold water; hot water from collector to tank; transparent cover; depletion tap; framework; collector; absorbing plate.

The tank isolated from top; auxiliary heater; hot water to house; input cold water; collectors placed in the ceiling.

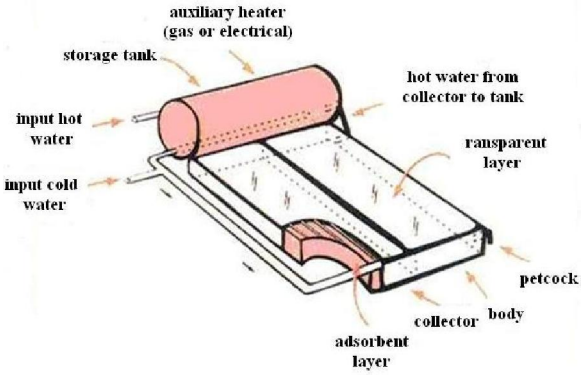

Fig 7- Closed-cycle thermo siphon system

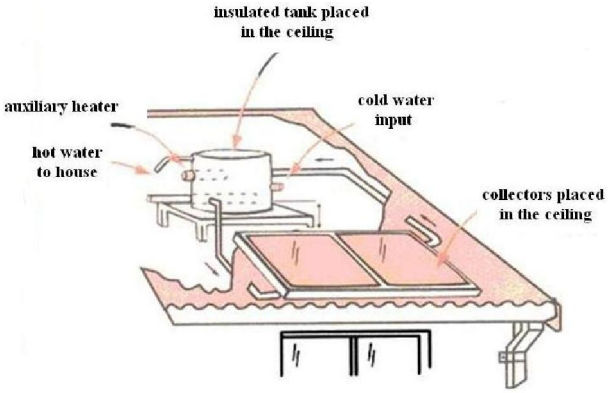

Fig 8- Thermo siphon system with separate tank

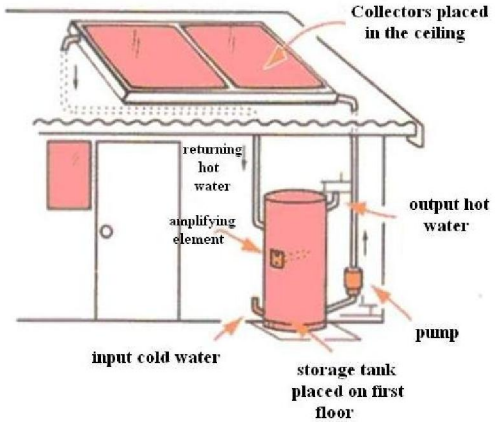

Fig 9- Compulsory circulating system

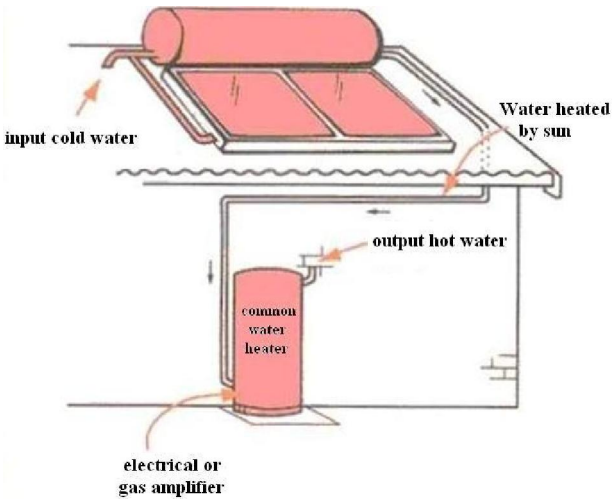

Fig 10- Pre-heater with a closed-cycle solar system

8. Vision of using photovoltaic systems

Common fuel resources which are non-reproducible have caused numerous environmental problems such as increase in earth temperature, acidic rains, water pollution, rapid increase of garbage, corruption of environment, and waste of natural resources. The photovoltaic systems however cause none of these negative environmental problems. The raw material for making the photovoltaic modules is silicon, which is abundantly available. During its lifetime, silicon cells obtained from one ton of sand can generate electricity equal to burning 500,000 tons of coal. Also, photovoltaic technology can create many jobs. Some of these occupations are directly related to manufacturing these systems, while numerous other jobs are indirectly related to it, e.g. in making glass, metal, wiring, electric activities, building accessory equipments, and architecture [6].

In a general evaluation, each 100 million dollars selling of photovoltaic products creates 3800 occupations. It is predicted that until 2010 use of solar energy doubles and till 2030 the market of its products reaches 100 billion dollars. What guaranties the development of this system in future is its being clean, reproducibility, and reliability. Moreover, the advantages of novel and reproducible energies are of great importance and some of these advantages are mentioned in following.

a) Compatibility with environment

The first and most obvious characteristic of these energy resources is that these types of energies are compatible with the environment. The heat energy caused by fossil fuels and nuclear energy make its specific environmental problems. It should be noted that the viewpoint of societies toward the energy resources compatible with the environment gets increasingly pore positive. For example, subsequent to increase in earth's temperature and after the activities following Kyoto protocol, some countries like India decided to perform wide range of activities in order to minimize the amount of greenhouse gases produced in these countries.

b) Environmental advantages

- Reproducible energies never produce CO2 or other gases which are polluting and harmful to atmosphere.
- Reproducible energies do not produce garbage and harmful remains.
- We never run out of these energies.

c) Strategic advantages

- Reproducible energy can be produced locally.
- These energies lead to energy independency.

d) Social and economical advantages

- Reproducible energies promote the level of small societies since their equipments are often installed in rural regions.
- These kinds of energies provide the opportunity of creation and development of national technologies for nations.

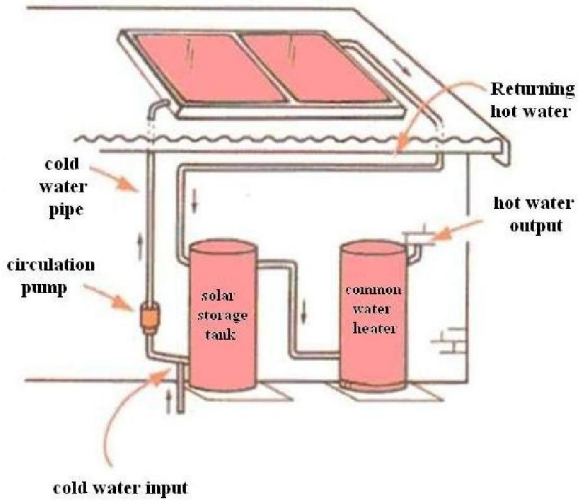

Fig 11- Pre-heater with solar storage tank on the ground

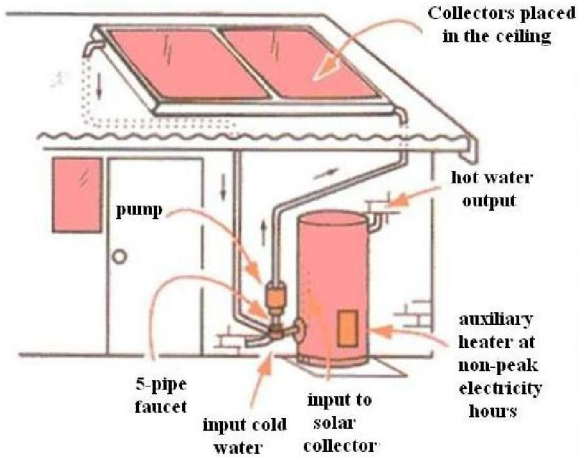

Fig 12- retrofits (electricity at non-peak hours)

9. Conclusion and suggestions

1- Clean and reproducible energies, including solar energy, have attracted all countries, both developing and developed ones, due to their numerous advantages regarding energy providing and decrease in air pollution. We hope that more attention will be paid to application of reproducible energies which results in stable development, saving people's lives, and service to human society, so that we will observe less victims during earthquake occurrence.

2- When earthquake happens and urban electricity transfer is not possible, we can make use of photovoltaic systems because they generate electricity independently and require no electricity transfer line and continuous maintenance. Considering the built-in modules in these systems, electricity can be generated in different scales. For instance, if we utilize this system as small individual modules, it satisfies our small needs. On the other hand, if a set of wide photovoltaic arrangements is used, we have established an enormous power plant for operation.

3- One of the most significant challenges is that the largest portion of society has little knowledge of the solar energy applications and no favorite cultural activities have been performed.

4- Suggestions have been offered to all of municipality regarding the establishment of earthquake solar parks and if these suggestions are applied, people will experience the application of these energies in a concrete way.

5- Establishment of solar cities is another suggestion which can solve lots of problems in this field and if this suggestion is implemented, we will observe a considerable transition in urbanism. As an example, Saudi Arabia has now three solar cities and all of the required energy of these cities is provided via solar energy.

6- In a system of non-reproducible resources, the costs of material transportation and labor are very high, but no such costs are present in production cycle of photovoltaic systems. Furthermore, through programming and executing short-term and long-term plans in the field of using solar energy, the opportunity is offered to make use of oil, this valuable black gold which is considered to provide the development and promotion of nations, in production of thousands of petrochemical products.

7- Without any doubt, one of the most important activities of advanced countries in the field of decreasing the consumption of non-clean energies is to help develop the technologies through which, utilization of reproducible and unlimited energy resources will be possible. Reaching this objective and accomplishing this goal will have a great influence on environment and economics of the countries. In photovoltaic systems, there is no need to resources of fossil fuel, so the environmental disadvantages caused by these resources and also cost of transportation and storekeeping are removed. In addition, photovoltaic systems are immobile and soundless and produce no audio noise.

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3/5/2011

Comparative Studies on Some Factors Affecting Rooting Ability of Carob Stem Cuttings

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Abstract: The present study was carried out during two successive seasons of 2007 and 2008 on 20 – years –old on a Carob (*Ceratonia Silique L.*), female tree which performed the best vegetative growth ,yield and pod quality, grown in the experimental orchard station of Horticulture Research Institute at Giza, Egypt .The aim was to find out the effect of some factors (type of cutting, date of cutting preparation and hormone treatment) on vegetative propagation of Carob plants by stem cuttings. To achieve this work, two types of Carob stem cuttings (terminal and sub-terminal) were prepared from one- year- old branches. Monthly, on each collection date, cuttings were subjected to the hetero auxin (IBA) treatments by dipping the base of them 10 sec. in 3000, 6000 and 9000 ppm IBA solution, with or without wounding and with or without NAA at two concentrations 100 & 200 ppm. Cuttings were divided into two groups; first group was planted under intermittent mist, and the second group under white plastic tunnel. Results showed that rooting ability of carob, root length and dry weight, survival after 2 months and average number of roots increased by increasing concentration of IBA from 3000 to 9000 ppm with wounding and with NAA at 100 and 200 ppm in both April and May in both terminal and sub terminal cuttings, but decreased to the little extent in August and September. While, fluctuated greatly exhibiting very low values to zero during the period from October to March. Terminal cuttings were the best in the rooting ability, root length and survival after 2 months. Sub-terminal cuttings were the best in the average number of roots and dry weight of roots under mist and tunnel. The untreated Carob stem cuttings of two types appeared to be difficult or impossible to root in collection dates and different concentrations of hormone. In conclusion, vegetative propagation of Carob by cuttings under mist or under tunnel affected by many factors such as: time of preparing cuttings, type of cuttings and treated with growth regulators. Moreover, mist propagation technique costly where as in areas characterized by poor facilities, propagation under white plastic tunnels is cheaper and save the cost of electricity and can be used commercially.
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http://www.americanscience.org.

Key words: Carob tree,type of cutting, date of cutting preparation and hormone treatment, stem cutting types

1. Introduction

Carob is native to the eastern Mediterranean, probably the Middle East, where it has been in cultivation for at least 4000 years. Carob grows well in the Mediterranean basin, and some parts of Western Asia and the Middle East. The tree is well adapted to mild and dry areas with poor soils. Carob tree is an important part of Mediterranean vegetation and it is often interplant with olives, grapes, almonds, and barley in low intensive faming system. *(Hill Coat et al., 1980*).

Carob trees may be male, female and hermaphrodite or play gummous inflorescences, showing high plasticity in inflorescences and flowering characteristics. Female cultivars are the most important trees commercial groves of Mediterranean countries. *Laubscher and Ndakidemi,(2008*) .

The carob is cultivated primarily in Mediterranean countries; most of the world's carob production comes from Mediterranean countries. Portugal and Spain have approximately 100,000 has of carob trees and process approximately half of the world's commercial carob supply. World carob pod production is approximately 315,000 t per year and the main carob bean producers and exporters are Spain (42%), Italy (16%), Portugal (10%), Morocco (8%), Greece (6.5%), Cyprus (5.5%) and Turkey (4.8%) (*Haselberg 2000*).

Carob is drought-resistant, requires little maintenance and produces a range of products from the seed and the pod. The endosperm is extracted from the seeds to produce a galactomannan, which forms locust bean gum .It is a valuable natural food additive used also in textile and cosmetic industries. The pod is useful for high-energy stock feed and the human food industry as a cocoa substitute and in syrups. Carob pod is also an anti-diarrheic product because of its high tannin content. (Loeb *et al.*, 1989).

Avallone et al., 1997 determined carob pod composition. High content of carbohydrates (45%), sucrose (30%), appreciable amounts of protein (3%) and low levels of fat (0.6%) were found. High tannin content is also present in carob pod composition, which limits the consumption by cattle because of reduced digestibility (*Priolo et al., 2000).*

Carob pods were also used in ancient Egypt, where the pulp of the pods was mixed in porridge, with a little

honey, and wax as a treatment for diarrhea and other. The trees are also useful as ornamentals and for landscaping, windbreaks and a forestation. Cattle can browse on leaves and the wood is suitable for fuel.

Carob has been neglected with respect to both cultural practices and research and development.

Carob grows well in a warm temperature and sub-tropical areas, tolerates hot and humid coastal areas. *(Roman et al., 2004)*

The art of propagation by cuttings is a very old technique popular in the field of horticulture. However, there are many species which are difficult to root. In recent years, many such difficults to root species are made to root easily by use of root inducing chemicals and

modifying the surrounding environment. Such attempts have been successful to get higher rooting percentage and early rooting. The rooting generally affected by internal and external factors i.e. type, date of taking and growth regulators application *(Hartman et al., 2002, Manan et al., 2002, Gerakakis and Ozkaya , 2005, Laubscher and Ndakidemi, 2008, Mohy 2009 and Sayed et al., 2010)* .

Plastic tunnels with water mist gave the best rooting and vegetative growth followed by the cutting under plastic tunnels without mist in Hamelia patens, (*Elgimabi, 2009).*

Carob may be propagated by seeds, budding, grafting or cutting *(Roman et al., 2002),* and has been described by *Lee et al. (1977)* and *Hartman and Kester (1983)* as a difficult to root. *Alorda er al.,(1987) and Cabrita et al., (1988)*

This investigation was carried out to study the effect of IBA & NAA concentration, wounding, time of cutting collection and the way of planting cuttings (mist or tunnels) on the rooting ability of two types (terminal or sub-terminal) of Carob cuttings.

2. Materials and Methods

This investigation was conducted through two seasons of 2007 and 2008 on 20 – years –old on a Carob *(Ceratonia Silique L.)* female tree which performed the best vegetative growth ,yield and pod quality , grown in the experimental orchard station of Horticulture Research Institute at Giza, Egypt was used as a source of cuttings.

This investigation included two experiments:

The first experiment studied the effect of some factors on vegetative propagation of Carob by using stem cuttings under mist.

The second experiment studied the effect of the same factors on vegetative propagation of Carob by using stem cuttings under tunnel.

Both experiments included three factors:

1- **Type of cuttings**

Two types of cuttings: terminal and sub terminal cuttings were prepared from one-year-old branches. Cuttings were about 15-20 cm in length, 1 – 1.5 cm in diameter and with 4 – 6 buds. A basal cut was made just below a nod and all leaves were removed except two leaves left at the apex.

2- **Growth regulators**

The basal end of cuttings to about 2 cm was quickly dipped for 10 seconds in different solutions of IBA concentration (3000, 6000 and 9000 ppm) with / or without wounding, and with / or without NAA in two concentrations 100 and 200 ppm.

The tested treatments were arranged as follow:

1- Control (water)
2- IBA at 3000 ppm
3- IBA at 3000 ppm + wounding
4- IBA at 3000 ppm + wounding + NAA at 100 ppm
5- IBA at 3000 ppm + wounding + NAA at 200 ppm
6- IBA at 6000 ppm
7- IBA at 6000 ppm + wounding
8- IBA at 6000 ppm + wounding + NAA at 100 ppm
9- IBA at 6000 ppm + wounding + NAA at 200 ppm
10- IBA at 9000 ppm
11- IBA at 9000 ppm + wounding
12- IBA at 9000 ppm + wounding + NAA at 100 ppm
13- IBA at 9000 ppm + wounding + NAA at 200 ppm

3-**Date of cutting preparation**

All cuttings were prepared at monthly intervals during the period from January till December.

Then each type of cuttings was divided into two groups:

First group (The first experiment) Carob cuttings were planted to depth of 5 – 6 in plastic flats containing a mixture of peat moss and sand (2: 1, V/V). Planted flats were directly kept under intermittent mist for 12 weeks. Misting was applied according to seasonal and daily weather conditions, within a range of 5-15 seconds ON and 2.5- 5 min OFF; bottom heat system was used during winter months.

The second group (The second experiment) cuttings were planted in one litre black plastic container (1 cutting / bag). The containers were filled with media of sand and peat moss (2 : 1, V/V) under white plastic tunnels 80 micron in diameter and placed in shaded net-house about 65 percentage porosity.

Measurements

- Rooting ability: root length, numbers of roots per cuttings and dry weight of roots (gm.) were recorded after 3 months of planting.
- Survival percentage: It was estimated on the number of rooted cuttings that remained alive two months latter from recording the rooting measurements.

The experiment was arranged in split plot design. Each treatment was replicated three times, and each replicate represented by 10 cuttings.

All collected data were subjected to statistical analysis for each year according to procedure out lined by *Gomez and Gomez (1984)* and the LSD was used to compare the treatment mean.

3. Results and discussion

3.1. The first experiment:

The effect of some factors [time of cutting preparation (dates), concentration of growth regulators and cutting position (type)] on vegetative propagation of Carob by using stem cuttings under intermittent mist.

3.1.1- Rooting ability (%)

Data in Tables (1&2) represented that treated Carob stem cuttings of two types along the whole year from January till December. It is evident from data that cutting preparation dates were very important factor for rooting ability. It can be noticed that, all treated cuttings were done from Oct. to March exhibited very low values to reach zero, April and May had fluctuated greatly. The highest rooting percentage was in April (40.90 & 40.44) followed by May (37.02, 37.26) in both seasons. Then decreasing in rooting percentage from June till September. As for treated cuttings with growth regulators, it is obvious that all treatments had increasing influence on rooting percent of cuttings compared with control the differences reach up to level significant, high concentration of IBA (9000 ppm) with wounding plus NAA at 100 ppm had the maximum rooting percentage in both seasons (30.4&30.27). Regarding the types of cutting (terminal and sub terminal) ;the treated terminal cuttings had the higher value than the treated sub terminal cuttings in April, May, and June (48.87,48.55, 43.35,43.21, & 34.90,34.68) in both seasons, respectively. Concerning interaction factors, it can be concluded that the terminal cuttings treated by IBA 9000 ppm with wounding plus NAA at 100 ppm through April was achieved the highest values in both seasons (62.47 & 63.13).

These results were in agreement with those obtained by *Mostafa et al., (1979), Mohamed (1980)* and *Al Tury et al., (1999)* on Carob. They reported that, date of preparing cuttings, different concentrations of IBA affected greatly on the rooting ability of Carob. Other investigators had reported results similar to ours [*El-Nabawy et al., (1983) and* El-*Said et al., (1990])* on olive .

3.1.2- Root length (mm):

Data in Tables (3&4) revealed that, the highest significant values of root length were obtained during April and May in the first season (9.55, 9.63) and during May in the second season (9.13). Then the root length decreased from June till Sept regarding the types of cuttings the terminal .Cuttings tended to have statistically significant higher root length than sub terminal ones from April to Sept. (11.22,11.38,11.14,9.15,6.96,6.74 cm and 10.82,11.07,10.70,8.96,6.37,6.14 cm) in both seasons. As for growth regulators treatments data presented in table (3&4) showed that all treatments significantly increased the root length as compared with control, best treatment was IBA at 9000 ppm with wounding (9.59, 10.25 cm) following by IBA at 9000 ppm with wounding plus NAA at 200 ppm (9.09 , 9.53 cm) treatment in both seasons, respectively.

Concerning the interaction the analysis of variance indicated that there was a significant respond to the interaction between date of preparation cutting, type of cutting, and concentration of growth regulators. It was clear from the current study that the highest length of root was achieved through April in terminal cutting treated with IBA at 9000 ppm with wounding (13.33,12.57) in both seasons .

These results are in line with *Alorda et al., (1987)* and *Cabrita et al., (1988)* on Carob; *El-Nabawy et al, 1983* and *Ibrahim et al., 2009* on Olive. They all reported that number of roots affected greatly by many factors such date, concentration of hormone, and planting date.

3.1.3- Number of roots:

It can be noticed in Tables (5&6) that number of roots increments were more pronounced in April in both seasons (10.38, 9.31) following in descending order by May, June, July, August and Sept.. As for type of cutting, it was quite evident that treated sub terminal cuttings had higher records during April, May and June in the first season , however the data in the second season did not take the same trend. The average number of
roots were positively affected by growth regulators, treatment with IBA at 9000 ppm
with wounding plus NAA at 200 ppm was superior applications followed by the
treatment with IBA at 9000 ppm with wounding plus NAA at 100 ppm (10.25, 10.11& 9.69, 9.67) in both seasons, respectively . Regarding the interaction between factors it can be concluded that the sub terminal cuttings treated by IBA at 9000 ppm with wounding plus NAA at 200 ppm or IBA at 9000 ppm with wounding plus NAA at 100 ppm through April recorded the highest number of roots (16.00,14.00 & 16.00,14.00) in both seasons.

The obtained results were in agreement with those obtained by *Mohamed (1980)* and *Ramon et al., (2002)* on Carob, as well as, *Mencuccin et al., (1988)* on Olive and Mohy Eldeen *(2009)* on Jojoba

3.1.4-Dry weight of roots (gm):

Tables (7&8) displayed that the treated cuttings which prepared in April had the significantly highest dry weight (6.16 & 6.16 gm.) in both seasons following in descending order by May, June, July, August and Sept.. As for type of cuttings, it is clear from available data that significant differences were observed in dry weight of roots, where the treated sub terminal cuttings recorded higher values than terminals ones during the months of April, May and June (6.94, 6.09, 5.60, 6.96, & 6.07,5.60 gm.) in both seasons, respectively. Concerning the effect of different treatments on dry weight of roots it is obvious that cuttings treated with IBA at 9000 ppm with wounding plus NAA at 200 ppm or IBA at 9000 ppm with wounding plus NAA at 100 ppm had the highest values in this respect (5.93, 5.84 & 5.93, 5.82) in both seasons, respectively. Meanwhile, the interaction between factors was significantly affected dry weight of roots. In this respect when sub terminal cuttings treated with IBA at 9000ppm + wounding plus NAA at 100ppm or IBA at 9000 ppm with wounding plus NAA at 200 ppm through April gave the maximum values (8.96, 8.83 & 9.01, 8.98) in both seasons, respectively .

Similar results were obtained by Lee and Hackett (1977), Hartman and Kester (1983), Alorda et al., (1987), Roman and Martins (2002) and Ibrahim et al., (2009).

Table (1): Effect of planting date, type of cutting and treating with hormone on the rooting ability (%) under mist in 2007 season

Treatments	April		May		June		July		August		Sept.		Mean (A)
	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	37.70	22.37	31.67	22.10	24.70	20.23	0.00	13.90	0.00	0.00	0.00	0.00	14.39
3	48.13	29.83	37.63	26.50	27.00	23.20	12.17	15.40	0.00	0.00	0.00	0.00	18.32
4	51.67	29.17	39.58	26.17	31.40	22.83	13.00	16.07	0.00	0.00	0.00	0.00	19.16
5	44.57	29.00	35.87	25.70	26.47	22.57	11.40	14.73	0.00	0.00	0.00	0.00	17.53
6	42.23	33.63	35.00	29.63	23.50	24.17	13.67	14.07	6.87	12.83	5.07	9.93	20.88
7	45.87	36.23	45.80	32.87	34.90	26.90	16.20	15.83	11.83	14.27	6.47	10.87	24.84
8	51.20	35.40	49.37	31.53	37.20	25.40	13.70	15.73	8.70	14.40	5.30	11.00	24.91
9	46.65	33.83	40.58	31.53	31.70	25.03	12.20	15.70	8.37	13.33	4.40	10.00	22.78
10	48.83	37.07	44.67	35.87	33.83	28.63	11.83	15.00	11.67	13.00	6.37	9.83	24.72
11	51.50	35.17	49.83	37.20	44.30	33.03	18.10	12.17	13.03	14.97	7.67	10.00	27.50
12	62.47	38.40	57.50	34.73	54.93	31.43	19.27	16.17	14.57	15.27	8.53	10.17	30.04
13	55.63	35.13	52.70	34.33	48.90	30.33	14.37	14.50	11.20	14.07	7.60	9.07	27.32
Mean(B)	40.90		37.02		30.53		13.97		8.27		5.51		
Mean(C)	48.87	32.94	43.35	30.68	34.90	26.15	12.99	14.94	7.19	9.34	4.28	6.74	

L.S.D.	A	B	C	ABC
	0.3400	0.2404	0.1388	1.178

T1= IBA 3000 ppm
T2= IBA 3000 ppm + Wounding
T3= IBA 3000 ppm + Wounding + NAA 100 ppm
T4= IBA 3000 ppm + Wounding + NAA 200 ppm
T5= IBA 6 000 ppm
T6= IBA 6000 ppm + Wounding
T7= IBA 6000 ppm + Wounding + NAA 100 ppm
T8= IBA 6000 ppm + Wounding + NAA 200 ppm
T9= IBA 9000 ppm
T10= IBA 9000 ppm + Wounding
T11= IBA 9000 ppm + Wounding + NAA 100 ppm
T12= IBA 9000 ppm + Wounding + NAA 200 ppm

* (A): Growth regulators *(B): Cutting type *(C): Planting date ABC: Interaction

Table (2): Effect of planting date, type of cutting and treating with hormone on rooting ability (%) under mist in 2008 season.

Treatments	April		May		June		July		August		Sept.		Mean (A)
	Ter.	Sub ter.	Ter.	Sub	Ter.	Sub	Ter.	Sub	Ter.	Sub	Ter.	Sub	
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	37.27	22.10	31.22	21.93	24.67	20.00	0.00	13.90	0.00	0.00	0.00	0.00	14.26
3	44.48	30.27	37.20	27.60	27.67	23.33	11.57	16.23	0.00	0.00	0.00	0.00	18.20
4	51.10	29.70	39.50	27.70	30.53	22.67	12.67	16.40	0.00	0.00	0.00	0.00	19.19
5	44.40	28.80	35.57	26.67	26.33	23.07	11.10	14.93	0.00	0.00	0.00	0.00	17.57
6	42.50	27.57	35.53	31.23	23.23	24.50	13.40	14.33	7.03	13.10	5.00	9.67	20.59
7	46.37	35.83	46.50	34.00	34.33	27.00	16.23	16.43	11.73	14.50	7.20	10.87	25.08
8	51.10	34.37	48.43	31.87	37.00	25.73	13.53	15.97	8.70	14.67	5.13	10.53	24.75
9	46.54	33.67	40.55	32.17	31.63	25.33	11.97	15.50	8.33	13.10	4.60	9.90	22.77
10	48.83	37.50	43.64	36.50	33.57	25.10	11.50	15.30	11.60	13.83	6.40	9.57	24.44
11	51.23	35.17	49.67	37.63	44.33	28.83	17.73	16.63	13.13	15.43	7.67	10.43	27.58
12	63.13	38.50	57.67	35.00	54.50	33.27	18.87	16.20	14.77	14.97	8.60	10.73	30.27
13	55.60	34.57	53.07	33.33	48.33	31.07	14.77	14.83	11.17	13.97	7.57	9.10	27.28
Mean(B)	40.44		37.26		30.25		14.17		8.33		5.54		
Mean(C)	48.55	32.34	43.21	31.30	34.68	25.83	12.78	15.56	7.21	9.46	4.35	6.73	

L.S.D.	A	B	C	ABC
	0.3909	0.2764	0.1596	1.354

T1= 3000 ppm
T2= 3000 ppm + Wounding
T3= 3000 ppm + Wounding + NAA 100ppm
T4= 3000 ppm + Wounding + NAA 200ppm
T5= 6000 ppm
T6= 6000 ppm + Wounding
T7= 6000 ppm + Wounding + NAA 100ppm
T8= 6000 ppm + Wounding + NAA 200ppm
T9= 9000 ppm
T10= 9000 ppm + Wounding
T11= 9000 ppm + Wounding + NAA 100ppm
T12= 9000 ppm + Wounding + NAA 200ppm
* (A): Growth regulators *(B): Cutting type *(C): Planting date ABC: Interaction

Table (3): Effect of planting date, type of cutting and treating with hormone on length of roots under mist in 2007 season

Treatments	April		May		June		July		August		Sept.		Mean (A)
	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	10.40	6.67	10.57	6.67	10.17	6.63	0.00	6.17	0.00	0.00	0.00	0.00	4.77
3	11.50	8.00	11.67	7.83	11.17	7.47	10.60	7.10	0.00	0.00	0.00	0.00	6.28
4	10.50	7.40	10.50	7.57	10.17	7.20	9.40	6.30	0.00	0.00	0.00	0.00	5.75
5	10.27	7.33	10.33	7.30	10.17	6.83	8.97	6.23	0.00	0.00	0.00	0.00	5.62
6	10.90	7.50	11.17	7.47	11.00	7.60	10.17	6.67	10.17	6.10	10.17	6.07	8.75
7	12.00	8.50	12.33	8.40	12.33	8.47	10.50	7.07	11.17	6.30	10.83	6.40	8.63
8	10.83	8.00	10.73	7.80	11.67	8.10	9.33	6.53	10.17	6.10	9.00	6.00	8.69
9	10.07	7.67	10.40	8.00	10.50	7.67	9.17	6.20	9.83	6.00	9.07	5.67	8.35
10	11.83	8.30	12.73	8.30	12.17	7.50	10.83	7.00	11.00	6.63	10.67	6.17	9.04
11	13.33	8.83	14.00	8.80	13.00	8.40	11.67	7.50	11.83	7.17	11.67	6.83	10.25
12	11.76	8.20	11.33	8.50	11.00	8.10	9.83	7.00	9.83	6.83	10.00	6.17	9.45
13	11.33	8.10	10.83	8.00	10.40	7.83	9.33	6.37	9.50	6.37	9.50	6.00	9.53
Mean(B)	9.55		9.63		9.40		7.91		5.63		5.43		
Mean(C)	11.22	7.88	11.38	7.89	11.14	7.65	9.15	6.68	6.96	4.29	6.74	4.11	

L.S.D.	A	B	C	ABC
	0.1856	0.1312	0.07576	0.6428

T1= 3000 ppm
T2= 3000 ppm + Wounding
T3= 3000 ppm + Wounding + NAA 100ppm
T4= 3000 ppm + Wounding + NAA 200ppm
T5= 6000 ppm
T6= 6000 ppm + Wounding
T7= 6000 ppm + Wounding + NAA 100ppm
T8= 6000 ppm + Wounding + NAA 200ppm
T9= 9000 ppm
T10= 9000 ppm + Wounding
T11= 9000 ppm + Wounding + NAA 100ppm
T12= 9000 ppm + Wounding + NAA 200ppm
* (A): Growth regulators *(B): Cutting type *(C): Planting date ABC: Interactio

Table (4): Effect of planting date, type of cutting and treating with hormone on the length of roots under mist in 2008 season

Treatments	April		May		June		July		August		Sept.		Mean (A)
	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	9.83	6.57	10.57	6.33	10.27	6.23	0.00	6.27	0.00	0.00	0.00	0.00	4.67
3	11.13	7.73	11.17	7.60	11.27	7.17	10.43	7.10	0.00	0.00	0.00	0.00	6.13
4	10.67	7.20	10.33	7.47	10.57	7.17	9.47	6.17	0.00	0.00	0.00	0.00	5.75
5	9.83	7.17	10.23	7.27	9.97	7.07	9.00	6.10	0.00	0.00	0.00	0.00	5.55
6	10.83	6.80	10.67	7.30	10.27	7.27	10.17	6.20	9.57	6.07	9.10	6.03	8.36
7	11.30	7.43	11.67	8.20	11.57	8.07	10.67	7.17	10.50	6.37	9.83	6.30	7.89
8	10.50	7.03	10.83	7.00	10.53	6.67	9.00	6.80	9.00	6.07	8.93	6.10	8.21
9	10.00	6.83	10.53	6.50	9.40	6.40	8.40	7.03	8.10	6.00	8.27	5.63	7.76
10	11.47	7.40	12.17	7.17	11.57	6.87	10.10	7.23	10.07	6.53	9.60	6.30	8.87
11	12.57	7.87	13.17	7.67	12.40	7.60	11.07	7.60	10.67	7.13	10.57	6.83	9.59
12	11.00	7.47	11.30	7.30	10.50	7.20	10.17	7.03	10.00	6.83	9.17	6.67	8.72
13	10.67	6.87	10.17	6.57	10.10	6.17	9.07	6.37	8.57	6.70	8.23	6.23	9.09
Mean(B)	9.01		9.13		8.84		7.86		5.34		5.16		
Mean(C)	10.82	7.20	11.07	7.20	10.70	6.99	8.96	6.76	6.37	4.31	6.14	4.18	

L.S.D	A	B	C	ABC
	0.1415	0.1000	0.05776	0.4901

T1= 3000 ppm
T2= 3000 ppm + Wounding
T3= 3000 ppm + Wounding + NAA 100ppm
T4= 3000 ppm + Wounding + NAA 200 ppm
T5= 6000 ppm
T6= 6000 ppm + Wounding
T7= 6000 ppm + Wounding + NAA 100ppm
T8= 6000 ppm + Wounding + NAA 200 ppm
T9= 9000 ppm
T10= 9000 ppm + Wounding
T11= 9000 ppm + Wounding + NAA 100ppm
T12= 9000 ppm + Wounding + NAA 200 ppm

* (A): Growth regulators *(B): Cutting type *(C): Planting date ABC: Interaction

Table (5): Effect of planting date, type of cutting and treating with hormone on number of roots of Carob under mist in 2007 season

Treatments	April		May		June		July		August		Sept.		Mean (A)
	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	
1	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*
2	*3.67*	*7.67*	*4.00*	*5.67*	*4.33*	*5.33*	*2.67*	*3.00*	*0.00*	*2.67*	*0.00*	*2.33*	*3.44*
3	*4.67*	*10.33*	*5.33*	*9.00*	*6.00*	*4.33*	*5.33*	*4.33*	*0.00*	*3.00*	*0.00*	*2.33*	*4.56*
4	*6.33*	*11.33*	*6.67*	*10.00*	*6.33*	*8.00*	*7.00*	*5.33*	*0.00*	*3.00*	*0.00*	*2.67*	*5.56*
5	*7.33*	*12.67*	*7.67*	*11.33*	*7.33*	*8.67*	*8.00*	*6.00*	*0.00*	*3.00*	*0.00*	*3.00*	*6.25*
6	*8.33*	*8.33*	*8.67*	*7.33*	*8.00*	*9.00*	*5.67*	*3.33*	*5.67*	*4.00*	*4.33*	*2.67*	*6.28*
7	*9.00*	*12.00*	*7.00*	*11.00*	*8.33*	*9.67*	*7.67*	*4.67*	*8.67*	*4.67*	*6.00*	*3.00*	*7.64*
8	*10.67*	*13.33*	*8.67*	*11.00*	*8.67*	*10.00*	*8.67*	*5.67*	*9.33*	*5.00*	*8.00*	*3.33*	*8.53*
9	*10.00*	*13.67*	*9.33*	*12.33*	*9.33*	*11.33*	*9.00*	*6.00*	*9.67*	*5.67*	*8.67*	*3.67*	*9.06*
10	*9.33*	*9.67*	*9.67*	*8.00*	*8.67*	*7.00*	*8.00*	*6.33*	*8.67*	*5.67*	*6.00*	*4.33*	*7.61*
11	*10.33*	*14.33*	*11.00*	*11.67*	*9.67*	*9.67*	*9.00*	*6.67*	*8.67*	*6.00*	*8.33*	*4.33*	*9.14*
12	*11.00*	*16.00*	*11.33*	*11.67*	*10.33*	*10.67*	*9.33*	*7.33*	*8.67*	*6.33*	*9.00*	*4.67*	*9.69*
13	*13.00*	*16.00*	*12.33*	*12.00*	*11.00*	*11.33*	*9.67*	*8.00*	*8.67*	*6.67*	*9.33*	*5.00*	*10.25*
Mean (B)	*10.38*		*9.28*		*8.46*		*6.53*		*5.15*		*4.21*		
Mean (c) C)	*8.64*	*12.11*	*8.47*	*10.08*	*8.17*	*8.75*	*7.50*	*5.56*	*5.67*	*4.64*	*4.97*	*3.44 I*	

L.S.D.	*A*	*B*	*C*	*ABC*
	0.4126	*0.2918*	*0.1684*	*1.429*

T1= 3000 ppm
T2= 3000 ppm + Wounding
T3= 3000 ppm + Wounding + NAA 100ppm
T4= 3000 ppm + Wounding + NAA 200ppm
T5= 6000 ppm
T6= 6000 ppm + Wounding
T7= 6000 ppm + Wounding + NAA 100ppm
T8= 6000 ppm + Wounding + NAA 200ppm
T9= 9000 ppm
T10= 9000 ppm + Wounding
T11= 9000 ppm + Wounding + NAA 100ppm
T12= 9000 ppm + Wounding + NAA 200ppm

*** (A):** Growth regulators ***(B):** Cutting type ***(C):** Planting date **ABC:** Interac

Table (6): Effect of planting date, type of cutting and treating with hormone on number of roots of Carob under mist in 2008

Treatments	April		May		June		July		August		Sept.		Mean (A)
	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	4.67	6.67	5.00	5.33	4.67	3.00	0.00	3.00	0.00	2.33	0.00	2.33	3.08
3	5.67	9.00	6.00	8.00	5.67	5.33	5.00	4.67	0.00	2.67	0.00	2.67	4.56
4	6.33	9.67	6.67	9.00	6.67	6.00	6.67	5.33	0.00	2.67	0.00	2.67	5.14
5	7.33	10.67	7.33	10.00	7.33	6.67	7.33	5.67	0.00	2.67	0.00	2.67	5.64
6	5.33	6.33	5.67	5.33	6.00	7.33	6.33	3.33	5.33	3.00	4.00	3.00	5.08
7	6.67	10.00	7.00	9.00	7.00	8.00	8.00	5.00	7.67	3.67	5.33	3.33	6.72
8	8.33	11.33	8.00	9.00	8.00	8.00	9.00	5.00	8.33	4.33	7.00	3.33	7.47
9	9.00	11.67	9.00	10.33	9.00	9.33	10.00	6.00	9.00	5.00	8.00	3.67	8.33
10	8.33	8.33	9.00	6.00	6.00	5.00	8.33	6.67	7.33	5.33	6.00	3.33	6.64
11	11.67	12.33	12.00	9.67	9.33	8.33	9.33	5.00	8.67	6.00	8.00	4.00	8.69
12	12.67	14.00	13.00	9.67	10.67	8.67	10.33	7.33	9.67	6.67	9.00	4.33	9.67
13	13.67	14.00	13.33	10.00	11.67	9.33	10.33	7.67	10.00	7.00	9.67	4.67	10.11
Mean (B)	9.31		8.49		7.38		6.47		4.89		4.04		
Mean C)	8.28	10.33	8.53	8.44	7.67	7.08	7.56	5.39	5.50	4.28	4.75	3.33	

L.S.D	A	B	C	ABC
	0.4443	0.3141	0.1814	1.539

T1= 3000 ppm T2= 3000 ppm + Wounding T7= 6000 ppm + Wounding + NAA 100ppm T8= 6000 ppm + Wounding + NAA 200ppm

T3= 3000 ppm + Wounding + NAA 100ppm T4= 3000 ppm + Wounding + NAA 200ppm T9= 9000 ppm T10= 9000 ppm + Wounding

T5= 6000 ppm T6= 6000 ppm + Wounding T11= 9000 ppm + Wounding + NAA 100ppm T12= 9000 ppm + Wounding + NAA 200ppm

* (A): Growth regulators *(B): Cutting type *(C): Planting date ABC: Interac

Table (7): Effect of planting date, type of cutting and treating with hormone on the dry weight of roots (gm.) under mist in 2007 season

Treatments	April		May		June		July		August		Sept.		Mean (A)
	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	2.88	5.48	3.15	3.34	3.25	3.95	0.00	2.65	0.00	0.00	0.00	0.00	2.06
3	3.24	6.31	3.38	5.79	4.17	4.97	3.82	3.00	0.00	0.00	0.00	0.00	2.89
4	4.52	6.79	4.58	6.29	4.41	5.17	5.00	3.80	0.00	0.00	0.00	0.00	3.38
5	5.06	6.90	5.19	6.73	5.12	5.30	5.21	4.37	0.00	0.00	0.00	0.00	3.66
6	5.16	5.23	5.23	5.38	5.07	5.36	4.13	2.58	4.33	3.15	3.88	2.08	4.02
7	5.69	6.58	5.02	6.55	5.19	5.51	5.06	3.17	5.22	4.15	4.01	2.31	4.87
8	6.16	7.03	5.15	6.54	5.20	6.17	5.14	4.00	5.78	4.40	5.36	2.89	5.32

Mean(A)	6.12	6.93	5.53	5.16	5.10	5.33	4.98	3.59	5.03	4.63	3.58	3.00
	6.29	7.07	6.33	6.95	4.97	5.89	4.75	5.47	4.91	4.15	4.80	3.19
	6.80	7.96	6.49	6.88	6.26	6.43	5.91	5.06	4.93	4.17	5.00	3.18
	7.12	8.83	6.95	6.74	6.59	6.81	5.32	5.12	5.07	4.28	5.09	3.29
Mean(B)	6.16		5.62		5.30		4.21		3.06		2.45	
Mean(C)	6.94	5.37	5.16	6.09	5.00	5.60	4.48	3.94	3.32	2.79	3.01	1.89

L.S.D	A	B	C	ABC
	0.08036	0.05682	0.0328	0.2784

T1= 3000 ppm T2= 3000 ppm + Wounding T7= 6000 ppm + Wounding + NAA 100ppm T8= 6000 ppm + Wounding + NAA 200ppm

T3= 3000 ppm + Wounding + NAA 100ppm T4= 3000 ppm + Wounding + NAA 200ppm T9= 9000 ppm T10= 9000 ppm + Wounding

T5= 6000 ppm T6= 6000 ppm + Wounding T11= 9000 ppm + Wounding + NAA 100ppm T12= 9000 ppm + Wounding + NAA 200ppm

* (A): **Growth regulators** *(B): **Cutting type** *(C): **Planting date** ABC: **Interac**

Table (8): Effect of planting date, type of cutting and treating with hormone on the dry weight of roots (gm.) under mist in2008 season

Treatments	April		May		June		July		August		Sept.		Mean (A)
	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	2.97	5.28	3.17	3.30	3.21	4.08	0.00	2.70	0.00	0.00	0.00	0.00	2.06
3	3.17	6.48	3.36	5.79	4.14	5.07	3.79	3.11	0.00	0.00	0.00	0.00	2.91
4	4.42	6.68	4.51	6.26	4.41	5.11	5.09	3.83	0.00	0.00	0.00	0.00	3.36
5	5.02	6.75	5.17	6.69	5.08	5.31	5.21	4.34	0.00	0.00	0.00	0.00	3.63
6	5.13	5.28	5.10	5.31	5.11	5.31	4.17	2.45	4.32	3.18	3.77	2.09	4.27
7	5.65	6.68	5.02	6.52	5.20	5.60	5.23	3.11	5.23	4.21	4.01	2.30	4.90
8	6.22	7.20	5.19	6.68	5.16	6.22	5.17	4.10	5.88	4.34	5.28	2.81	5.35

Mean(A)	6.05	7.18	4.83	6.76	4.70	6.21	4.58	4.53	4.57	4.63	4.43	2.74	5.10
	5.66	6.05	5.47	5.15	5.06	5.29	5.06	3.69	5.01	4.50	3.38	3.07	4.78
	6.23	7.98	6.24	6.92	4.93	5.83	4.77	5.48	5.06	4.09	4.78	3.18	5.46
	6.72	9.01	6.45	6.82	6.26	6.40	5.83	5.00	4.86	4.20	5.11	3.20	5.82
	7.00	8.98	6.83	6.68	6.65	6.82	5.28	5.09	5.10	4.30	5.14	3.30	5.93
Mean(B)	6.16		5.59		5.30		4.23		3.06		2.44		
Mean(C)	5.35	6.96	5.11	6.07	4.99	5.60	4.52	3.95	3.34	2.79	2.99	1.89	

L.S.D	A	B	C	ABC
	0.04866	0.03441	0.01986	0.1686

T1= 3000 ppm T2= 3000 ppm + Wounding T3= 3000 ppm + Wounding + NAA 100ppm T4= 3000 ppm + Wounding + NAA 200ppm T5= 6000 ppm

T6= 6000 ppm + Wounding
T7= 6000 ppm + Wounding + NAA 100ppm
T8= 6000 ppm + Wounding + NAA 200ppm
T9= 9000 ppm
T10= 9000 ppm + Wounding
T11= 9000 ppm + Wounding + NAA 100ppm
T12= 9000 ppm + Wounding + NAA 200ppm
* (A): **Growth regulators** *(B): **Cutting type** *(C): **Planting date** ABC: **Intera**3.1.5- Survival after 2 months (%).

Data of statically evaluation of the percentage of survival cuttings, was illustrated in Tables (9&10). It indicated that treated carob cuttings at April and May were significantly achieved the highest values in this respect (80.60, 80.80&80.19, 89.89), (80.47,77.92,80.52,79.76) in both season respectively then the percentage of survival cuttings decreased from June till Sept.. Concerning the type of cutting the treated terminal and sub-terminal Carob cuttings significantly attained the maximum survival cuttings (80.56& 80.65 &80.98, 80.62), but in the second season the treated terminal cuttings significantly gave the higher value than sub terminal treated cuttings through April and may. As growth regulators; it can be said that terminal Carob cuttings treated with IBA at 9000 ppm plus wounding during April in the first season , while in the second season the same types of cuttings and month but treated with IBA at 6000ppm plus wounding NAAat2000 ppm were the superior in this treatment respect. Afterwards, the survival percentage of continuously decreased for both types of cuttings reaching the minimum value in August and Sept. (42.28, 37.98 & 42.69, 38.31) in both seasons, respectively. Meanwhile, the interaction among the factors under study was significantly affected on survival cuttings (%). In this respect when the terminal or sub terminal cuttings treated with IBA at 9000 ppm plus wounding significantly gave the highest values during April and May in both seasons.

These results were in agreement with the findings of *Mohamed (1980), El-Nabaway (1983), Alorda et al., (1987), Roman and Martin (2002), Ibrahim et al., (2009) and Luqman (2004)).*

Table (9): Effect of planting date, type of cutting and treating with hormone on survival (%) after 2 months under mist in 2007 season

Treatments	April		May		June		July		August		Sept.		Mean (A)
	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	78.20	81.30	82.40	83.57	78.80	79.90	0.00	71.27	0.00	0.00	0.00	0.00	46.29
3	79.87	79.73	81.90	81.47	80.00	79.50	73.00	75.30	0.00	0.00	0.00	0.00	52.56
4	79.33	80.87	83.00	81.40	74.90	76.10	67.00	75.67	0.00	0.00	0.00	0.00	51.52
5	79.90	79.90	78.87	78.20	79.87	75.07	64.30	74.03	0.00	0.00	0.00	0.00	50.84
6	80.28	78.53	80.63	79.07	80.70	75.07	70.23	77.43	65.83	63.67	55.27	55.37	71.84
7	81.30	80.37	82.47	83.47	77.90	78.20	74.23	76.57	65.77	66.67	56.40	58.23	73.46
8	77.70	81.03	80.50	82.43	76.30	79.40	70.97	65.13	64.17	55.00	50.53	53.63	69.73

9	81.37	79.83	78.60	80.40	74.33	75.57	71.57	72.87	61.70	57.30	53.00	53.70	70.02
10	82.97	79.83	81.60	81.33	77.43	73.03	75.33	77.07	67.73	61.20	55.67	60.53	72.81
11	83.08	83.27	80.70	79.47	80.57	74.70	75.57	77.83	68.87	66.47	63.97	61.00	74.46
12	81.33	82.33	79.67	79.60	79.20	78.80	71.03	75.53	64.80	70.60	58.40	60.07	73.45
13	81.37	80.80	81.47	77.03	77.27	74.83	67.53	74.27	56.77	58.10	57.97	57.77	70.60
Mean(B)	80.60		80.80		77.39		69.74		42.28		37.98		
Mean(C)	80.56	80.65	80.98	80.62	78.11	76.68	65.06	74.41	42.97	41.58	37.60	38.36	

L.S.D.	A	B	C	ABC
	0.7466	0.5279	0.3048	2.586

T1 = 3000 ppm
T2 = 3000 ppm + Wounding
T3 = 3000 ppm + Wounding + NAA 100 ppm
T4 = 3000 ppm + Wounding + NAA 200ppm
T5 = 6000 ppm
T6 = 6000 ppm + Wounding
T7 = 6000 ppm + Wounding + NAA 100 ppm
T8 = 6000 ppm + Wounding + NAA 200ppm
T9 = 9000 ppm
T10 = 9000 ppm + Wounding
T11 = 9000 ppm + Wounding + NAA 100 ppm
T12 = 9000 ppm + Wounding + NAA 200ppm
* (A): **Growth regulators** *(B): **Cutting type** *(C): **Planting date** ABC: **Inter**

3.2.The second experiment:

The effect of some factors [time of cutting preparation (dates), concentration of growth regulators and cutting position (type)] on vegetative propagation of Carob by using stem cuttings under tunnel

3.2. 1- Rooting ability (%)

With regard to date of cutting preparation data generally showed highly significant differences in rooting ability among treated carob cuttings at different months. It can be clearly noticed in Tables (11&12) that all treated cuttings were done from Oct. to March failed to root in both seasons, while April and May were the proper time for preparing the treated cuttings (51.22, 46.85 & 50.79, 47.53) then rooting percent was gradually decreased from June to Sept..(18.26, 7.14, 5.79, 4.52 & 19.74, 8.39, 6.47, 5.59) in both seasons, respectively. As for type of Carob cuttings, significant differences were observed in rooting percent of cuttings. It was clear that treated terminal cuttings showed in general higher rooting percent than the sub terminal ones from April until Sept. in both seasons. Regarding the effect of different treatments on rooting percentage of each type of cuttings, it is obvious that all treatments induced the great increasing influence on rooting percentage compared with control except months from October to March which treated cutting failed to root, where, the differences reached up to significant level. Best treatments were IBA at 9000ppm + wounding or IBA at 9000 ppm with wounding plus NAA at 100 ppm (26.93, 36.83 &37.78, 28.08) respectively, in both seasons. Concerning the interaction it can be noticed that the treated

terminal cuttings with IBA at 9000 ppm with wounding plus NAA at 100 during April achieved the highest values (84.37, 85.07) respectively, in both seasons, Mohy Eldeen (2009).

Table (10): Effect of planting date, type of cutting and treating with hormone on the survival (%) after 2 months under mist in2008 season

	A		Ma		J		J		Au		S		
	Ter.	Sub ter.		Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	
	78.87	79.37		81.20	79.37	80.50	0.00	72.43	0.00	0.00	0.00	0.00	65.85
	78.87	79.73		80.93	80.73	80.17	73.60	73.70	0.00	0.00	0.00	0.00	52.38
	78.87	78.30		80.33	75.60	76.77	67.80	74.73	0.00	0.00	0.00	0.00	51.15
	78.97	78.17		76.37	81.40	75.87	65.13	73.43	0.00	0.00	0.00	0.00	50.63
	82.18	77.37		78.23	81.47	75.07	70.80	76.60	67.17	64.33	55.73	56.03	72.33
	82.50	82.10		82.97	78.80	79.20	72.97	74.73	66.43	67.00	56.80	58.57	73.57
	79.33	82.97		81.43	78.23	80.03	72.20	63.33	65.17	54.50	51.20	53.63	70.16
	83.63	80.03		79.93	75.57	74.97	72.10	71.53	62.33	58.00	54.00	52.70	70.25
	80.27	78.27		80.67	78.60	72.03	73.93	74.33	67.07	61.97	56.47	61.50	72.08
	82.28	82.90		79.33	80.10	73.33	74.93	78.30	69.80	67.20	61.70	63.00	75.32
	77.97	81.37		79.13	79.13	79.10	71.63	76.53	63.30	70.60	59.30	60.70	73.15
	81.90	79.47		76.53	76.30	75.83	69.20	74.80	60.77	58.97	58.97	59.17	71.03
	80.19		89.89		77.84		69.53		42.69		38.31		
	80.47	79.92	100.02	79.76	78.78	76.91	65.36	73.71	43.50	41.88	37.85	38.78	
	0.6233		0.4407		0.2545		2.159						

T1= 3000 ppm T2= 3000 ppm + Wounding
T3= 3000 ppm + Wounding + NAA 100ppm T4= 3000 ppm + Wounding + NAA 200ppm
T5= 6000 ppm T6= 6000 ppm + Wounding
T7= 6000 ppm + Wounding + NAA 100ppm T8= 6000 ppm + Wounding + NAA 200ppm
T9= 9000 ppm T10= 9000 ppm + Wounding
T11= 9000 ppm + Wounding + NAA 100ppm T12= 9000 ppm + Wounding + NAA 200ppm
* (A): **Growth regulators** *(B): **Cutting type** *(C): **Planting date** ABC: **Inte**

Table (11): Effect of planting date, type of cutting and treating with hormone on the rooting ability (%) under tunnel in 2007 season.

Treatments	April		May		June		July		August		Sept.		Mean (A)
	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	49.13	24.03	48.70	23.13	20.43	7.33	4.33	5.50	4.13	0.00	3.83	0.00	15.88
3	55.27	31.57	51.20	30.17	26.80	8.00	6.40	6.10	6.03	0.00	5.83	0.00	18.95
4	62.13	30.10	53.27	29.93	26.97	7.07	6.37	5.50	5.37	0.00	5.47	0.00	19.35
5	57.27	29.53	51.40	28.83	22.67	6.97	5.03	5.20	4.30	0.00	5.37	0.00	18.05I
6	62.93	35.70	60.10	32.90	22.70	10.37	8.00	6.10	6.07	4.50	5.73	4.47	21.63
7	71.17	37.27	61.50	35.70	24.73	11.57	9.13	6.63	9.13	5.53	7.63	4.33	23.69
8	75.47	37.33	64.20	33.90	25.80	10.07	8.83	5.60	8.67	5.47	5.00	4.07	23.70
9	68.47	35.00	62.10	35.03	24.87	10.00	8.50	5.40	8.40	5.53	5.20	4.00	22.71
10	73.20	38.00	66.10	37.23	24.83	14.50	8.73	6.27	9.20	5.00	6.80	4.03	24.49
11	77.33	39.03	68.10	38.40	28.70	17.07	13.47	7.67	12.97	5.83	8.90	4.70	26.93
12	84.37	39.07	71.13	37.17	30.00	15.23	11.23	5.53	11.23	6.33	7.53	4.17	26.83

13	79.33	37.13	67.33	36.13	26.63	15.03	10.33	5.57	9.77	5.43	7.37	3.93	25.33
Mean(B)	51.22		46.85		18.26		7.14		5.79		4.52		
Mean(C)	68.01	34.43	60.43	33.26	25.43	11.10	8.36	5.92 I	7.94	3.64	6.22 I	2.81	

L.S.D.	A	B	C	ABC
	0.3201	0.2263	0.1307	1.109

T1= 3000 ppm T2= 3000 ppm + Wounding
T3= 3000 ppm + Wounding + NAA 100ppm T4= 3000 ppm + Wounding + NAA 200ppm
T5= 6000 ppm T6= 6000 ppm + Wounding
T7= 6000 ppm + Wounding + NAA 100ppm T8= 6000 ppm + Wounding + NAA 200ppm
T9= 9000 ppm T10= 9000 ppm + Wounding
T11= 9000 ppm + Wounding + NAA 100ppm T12= 9000 ppm + Wounding + NAA 200ppm
* (A): **Growth regulators** *(B): **Cutting type** *(C): **Planting date** ABC: **Int**

Table (12): Effect of planting date, type of cutting and treating with hormone on the rooting ability (%) under tunnel in 2008 season

Treatments	April		May		June		July		August		Sept.		Mean (A)
	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	50.00	23.67	49.17	23.23	28.63	7.33	6.53	5.70	6.67	0.00	6.07	0.00	17.25
3	36.37	31.57	51.43	30.33	27.30	8.03	8.80	6.17	8.63	0.00	8.03	0.00	18.06
4	62.33	29.50	53.80	29.53	28.30	7.00	10.70	5.63	7.63	0.00	7.40	0.00	20.15
5	66.23	28.97	51.73	28.47	24.53	7.03	9.50	5.17	7.67	0.00	7.23	0.00	19.71
6	63.37	35.17	61.23	32.63	23.53	10.40	9.93	6.40	7.27	4.47	6.50	4.50	22.12
7	73.07	37.73	64.40	35.83	26.27	11.33	11.63	6.40	8.90	5.60	8.63	4.37	24.51
8	75.93	35.50	65.30	34.17	28.30	10.10	11.87	6.00	8.53	5.57	8.10	4.57	24.49
9	69.70	35.00	62.67	35.00	25.80	10.23	10.33	5.33	8.07	5.40	7.17	4.00	23.23
10	72.77	37.60	67.63	37.17	29.17	14.70	10.73	6.33	10.93	6.07	10.23	4.17	25.63
11	77.87	37.30	68.97	39.37	31.57	17.17	15.27	6.93	14.83	6.50	11.10	5.10	27.78
12	85.07	38.63	74.67	37.20	35.83	15.40	13.20	6.23	12.13	5.37	10.33	4.27	28.08
13	79.03	36.70	70.93	35.77	30.70	15.10	10.87	5.80	10.00	5.00	8.53	3.97	26.03
Mean(B)	50.79		47.53		19.74		8.39		6.47		5.59		
Mean(C)	67.64	33.94	61.83	33.23	28.33	11.15	10.78	6.01	9.27	3.66	8.28	2.91	

L.S.D.	A	B	C	ABC
	0.9596	0.6785	0.3917	3.324

T1= 3000 ppm T2= 3000 ppm + Wounding
T3= 3000 ppm + Wounding + NAA 100ppm T4= 3000 ppm + Wounding + NAA 200ppm
T5= 6000 ppm T6= 6000 ppm + Wounding
T7= 6000 ppm + Wounding + NAA 100ppm T8= 6000 ppm + Wounding + NAA 200ppm
T9= 9000 ppm T10= 9000 ppm + Wounding
T11= 9000 ppm + Wounding + NAA 100ppm T12= 9000 ppm + Wounding + NAA 200ppm
* (A): Growth regulators *(B): Cutting type *(C): Planting date ABC: In3.2.2- Length of roots(mm)

Results illustrated in Tables (13&14) showed that, carob cuttings prepared in April and May had the highest values in this respect, as well as, terminal treated carob cuttings gave significantly longer roots than sub terminal cuttings. Length of roots for both types decreased from April to Sept. in both seasons. As for the specific effect of treatments, it can be noticed that, application of IBA at 9000ppm + wounding was the best ones (9.02 & 8.74) in both seasons. Regarding the interaction, the available data indicated that the terminal carob cuttings treated with IBA at 9000ppm + wounding in April gave the longest roots (12.17,10.27) in both seasons (9.43, 9.27*). Ibrahim et al.,(2009*) on olive

Table (13): Effect of planting date, type of cutting and treating with hormone on length of roots (cm.) under tunnel in 2007 season.

Treatments	April		May		June		July		August		Sept.		Mean (A)
	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	9.33	5.67	8.83	5.67	8.00	5.60	7.33	4.97	0.00	0.00	0.00	0.00	4.62
3	10.40	6.33	10.50	6.27	9.43	6.37	8.50	5.67	0.00	0.00	0.00	0.00	5.29
4	9.67	5.77	9.20	5.77	9.03	5.77	7.67	5.60	0.00	0.00	0.00	0.00	4.87
5	9.53	5.40	9.17	5.30	8.37	5.10	7.83	5.20	0.00	0.00	0.00	0.00	4.66
6	9.67	6.23	9.67	6.00	8.39	5.73	9.00	5.23	8.67	5.17	8.67	5.00	7.32
7	10.67	6.73	10.50	6.60	8.83	6.17	10.17	5.83	10.00	5.40	9.70	5.50	7.63
8	10.00	6.43	9.67	6.50	9.50	6.17	9.17	5.47	9.17	5.07	9.17	5.10	7.60

9	10.00	6.40	9.83	6.20	9.33	5.53	9.00	5.17	9.57	4.90	8.53	5.00	7.47
10	10.83	6.83	10.67	6.43	9.50	5.90	10.50	5.53	9.83	5.47	9.00	5.50	8.00
11	12.17	7.43	11.67	7.00	9.67	6.60	11.17	6.23	10.50	6.03	10.53	5.90	8.74
12	9.83	6.70	10.33	6.53	9.83	6.10	10.17	5.40	8.83	5.20	9.67	5.33	7.83
13	10.17	6.37	10.10	6.10	10.17	5.43	10.07	5.07	8.87	5.13	9.40	4.73	8.06
Mean(B)	8.03		8.10		7.18		7.33		4.91		4.86		
Mean(C)	10.19	6.36	10.01	6.20	9.26	5.87	9.21	5.45	6.29	3.53	6.22	3.51	

L.S.D.	A	B	C	ABC
	0.1587	0.1122	0.06478	0.5497

T1= 3000 ppm T2= 3000 ppm + Wounding
T3= 3000 ppm + Wounding + NAA 100ppm T4= 3000 ppm + Wounding + NAA 200ppm
T5= 6000 ppm T6= 6000 ppm + Wounding
T7= 6000 ppm + Wounding + NAA 100ppm T8= 6000 ppm + Wounding + NAA 200ppm
T9= 9000 ppm T10= 9000 ppm + Wounding
T11= 9000 ppm + Wounding + NAA 100ppm T12= 9000 ppm + Wounding + NAA 200ppm
* (A): **Growth regulators** *(B): **Cutting type** *(C): **Planting date**

Table (14): Effect of planting date, type of cutting and treating with hormone on length of roots (cm.) under tunnel in 2008 season.

Treatments	April		May		June		July		August		Sept.		Mean (A)
	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	8.33	6.83	8.23	7.00	8.17	6.67	0.00	6.17	0.00	0.00	0.00	0.00	4.28
3	9.47	7.97	9.80	8.00	9.50	7.33	7.17	7.00	0.00	0.00	0.00	0.00	5.52
4	9.00	7.47	8.50	7.50	8.83	7.27	8.50	6.23	0.00	0.00	0.00	0.00	5.28
5	8.90	7.33	8.27	7.50	8.33	6.90	7.50	6.23	0.00	0.00	0.00	0.00	5.08
6	9.47	7.73	8.67	7.33	8.77	7.33	7.33	6.37	7.50	6.07	7.53	6.00	7.51
7	9.50	8.53	9.83	7.67	9.50	8.47	9.00	7.00	9.83	6.47	9.67	6.33	8.28
8	9.57	8.03	8.33	8.60	8.83	8.13	10.00	6.70	9.40	6.07	9.20	5.97	8.24
9	9.67	8.00	8.50	8.17	8.40	7.83	9.00	6.33	9.20	6.00	9.17	5.73	8.00
10	9.17	8.50	10.47	8.43	9.67	7.50	8.17	7.00	8.20	6.60	8.33	6.40	8.20
11	10.27	8.83	11.00	8.90	11.00	8.40	9.57	7.67	9.50	7.17	9.57	6.83	9.02
12	10.00	8.37	10.00	8.47	10.17	8.00	9.17	7.17	8.87	6.67	8.50	6.83	8.52
13	9.77	8.39	9.67	8.00	9.27	7.70	8.83	7.10	8.47	6.40	8.40	6.33	8.48
Mean(B)	8.71		8.62		8.42		7.30		5.10		5.03		
Mean(C)	9.43	8.00	9.27	7.96	9.20	7.63	7.85	6.75	5.91	4.29	5.86	4.20	

L.S.D.	A	B	C	ABC
	0.1620	0.1146	0.06615	0.5613

T1= 3000 ppm T2= 3000 ppm + Wounding
T3= 3000 ppm + Wounding + NAA 100ppm T4= 3000 ppm + Wounding + NAA 200ppm
T5= 6000 ppm T6= 6000 ppm + Wounding
T7= 6000 ppm + Wounding + NAA 100ppm T8= 6000 ppm + Wounding + NAA 200ppm
T9= 9000 ppm T10= 9000 ppm + Wounding
T11= 9000 ppm + Wounding + NAA 100ppm T12= 9000 ppm + Wounding + NAA 200ppm
*** (A):** Growth regulators ***(B):** Cutting type ***(C):** Planting date

Table (15): Effect of planting date, type of cutting and treating with hormone on number of roots under tunnel in 2007 season

Treatments	April		May		June		July		August		Sept.		Mean (A)
	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	4.00	4.33	4.33	5.00	4.00	4.00	3.67	4.00	0.00	0.00	0.00	0.00	2.78
3	5.00	5.33	4.33	5.33	4.67	4.33	5.00	5.33	0.00	0.00	0.00	0.00	3.28
4	6.33	7.33	6.00	6.67	5.33	6.00	6.33	6.67	0.00	0.00	0.00	0.00	4.22
5	7.33	8.00	5.67	7.00	6.00	6.00	7.00	7.33	0.00	0.00	0.00	0.00	4.53
6	7.00	7.33	6.67	8.00	5.33	5.00	5.33	6.00	5.33	4.67	4.00	3.67	5.69
7	7.67	8.00	7.33	8.67	5.67	7.00	7.33	8.00	8.33	7.33	5.67	5.33	7.19
8	8.67	9.33	7.67	9.33	6.00	8.33	7.67	8.00	9.00	8.00	8.33	7.67	8.17
9	9.00	9.67	8.67	9.67	6.33	9.00	8.67	9.67	9.00	8.67	8.67	7.33	8.69
10	9.00	9.67	8.67	9.33	8.00	8.33	7.33	8.00	6.33	5.67	6.00	5.67	7.67
11	10.67	11.67	10.33	11.33	8.33	9.67	8.67	9.67	8.33	7.33	7.67	6.33	9.17
12	11.67	12.33	11.33	12.33	9.00	10.00	9.00	10.00	9.00	8.00	8.33	7.00	9.83
13	10.67	12.00	10.33	10.67	8.67	9.33	8.67	9.33	8.67	8.33	8.67	6.33	9.31
Mean (B)	8.42		8.11		6.85		7.36		5.08		4.44		
Mean C)	8.08	8.75	7.61	8.61	6.44	7.25	7.06	7.67	5.33	4.83	4.78	4.11	

L.S.D.	A	B	C	ABC
	0.3355	0.2372	0.1370	1.162

T1= 3000 ppm T2= 3000 ppm + Wounding
T3= 3000 ppm + Wounding + NAA 100ppm T4= 3000 ppm + Wounding + NAA 200ppm
T5= 6000 ppm T6= 6000 ppm + Wounding
T7= 6000 ppm + Wounding + NAA 100ppm T8= 6000 ppm + Wounding + NAA 200ppm
T9= 9000 ppm T10= 9000 ppm + Wounding
T11= 9000 ppm + Wounding + NAA 100ppm T12= 9000 ppm + Wounding + NAA 200ppm
* (A): **Growth regulators** *(B): **Cutting type** *(C): **Planting date**

Table (16): Effect of planting date, type of cutting and treating with hormone on number of roots under tunnel in 2008 season.

Treatments	April		May		June		July		August		Sept.		Mean (A)
	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	4.33	4.33	4.67	4.67	3.67	4.00	3.67	4.00	0.00	0.00	0.00	0.00	2.78
3	5.67	5.67	5.67	5.33	4.33	4.67	4.00	5.00	0.00	0.00	0.00	0.00	3.36
4	7.00	8.00	6.33	6.67	6.33	5.33	5.67	6.33	0.00	0.00	0.00	0.00	4.31
5	8.33	8.33	7.33	7.33	7.00	5.67	6.33	7.00	0.00	0.00	0.00	0.00	4.78
6	7.00	7.67	6.00	7.00	5.00	4.67	4.67	5.67	4.00	4.67	3.67	3.67	5.31
7	8.00	8.33	8.33	7.33	5.67	6.67	6.00	7.00	4.67	5.00	4.33	5.00	6.36
8	9.33	10.00	9.33	8.67	6.33	8.33	6.67	7.33	5.67	5.33	5.00	5.33	7.28
9	9.67	10.00	10.00	9.00	7.00	8.67	7.00	8.33	6.00	6.33	5.33	6.33	7.81
10	8.67	9.67	7.33	8.00	8.00	6.33	5.33	7.00	5.33	5.33	5.00	5.33	6.78
11	11.00	10.67	8.67	8.33	8.67	7.33	7.33	8.67	6.67	6.00	6.33	6.00	7.97
12	12.00	11.67	9.67	9.33	9.33	8.33	8.00	9.00	7.33	6.67	7.00	6.33	8.72
13	10.33	11.00	10.33	8.33	9.67	7.33	9.00	8.33	8.33	6.00	8.00	6.00	8.56
Mean(B)	**8.61**		**7.65**		**6.60**		**6.56**		**3.89**		**3.69**		
Mean(C)	8.44	8.78	7.81	7.50	6.75	6.44	6.14	6.97	4.00	3.78	3.72	3.67	

L.S.D.	A	B	C	ABC
	0.2721	0.1924	0.1111	0.9426

T1= 3000 ppm T2= 3000 ppm + Wounding
T3= 3000 ppm + Wounding + NAA 100ppm T4= 3000 ppm + Wounding + NAA 200ppm
T5= 6000 ppm T6= 6000 ppm + Wounding
T7= 6000 ppm + Wounding + NAA 100ppm T8= 6000 ppm + Wounding + NAA 200ppm
T9= 9000 ppm T10= 9000 ppm + Wounding
T11= 9000 ppm + Wounding + NAA 100ppm T12= 9000 ppm + Wounding + NAA 200ppm
*** (A):** Growth regulators ***(B):** Cutting type ***(C):** Planting date

3.2.3- Number of roots

The available data illustrated in Tables (15&16) showed the average of root number on treated carob cuttings as significantly affected by the preparation time, it can be seen that April and May were the proper time in this respect in both seasons . As for type of cutting, it was generally apparent that sub terminal treated cuttings gave higher number of roots than terminal cuttings through April May, June and July in first season but was not clear in the second season. Regarding the growth regulators there were significant difference. Among tested treatments, where, the best treatment was IBA at 9000ppm + wounding plus NAA at 100ppm in first season. But in the second one, treatment with IBA at 9000ppm + wounding plus NAA at 100ppm or IBA at 9000ppm + wounding plus NAA at 200ppm exhibited highest number of roots. Concerning interaction among factors data showed that sub terminal cuttings treated with IBA at 9000ppm + wounding plus NAA at 100ppm during April had the highest number of roots in the 1st season but in the 2nd one the treated terminal ones had the highest values Gerakakis and Ozkaya,.(2005).

3.2.4 Dry weight of roots (gm):

Data in Tables (17&18) indicated that maximum dry weight of roots was noticed in April following by May, then the value was gradually decreased from June to Sept. Concerning cutting type, the sub terminal carob cuttings recorded significant higher values than the terminal ones from April until July in the first season. But through April and May; only in the second season. As for treatment effect, it can be noticed that the cuttings treated with IBA at 9000ppm + wounding plus NAA at 100ppm recorded significant heaviest dry weight of roots followed by IBA at 9000ppm + wounding in both seasons. Regarding the interaction among factors, the highest were obtained from sub terminal cuttings treated with IBA at 9000ppm + wounding plus NAA at 100ppm in April and May in both seasons. Elgimabi (2009).

3.2.5- Survival after 2 months (%):

The percentage of survival cuttings after 2 months for both terminal and sub-terminal cutting at the different collection dates from January to December in 2007 and 2008 are shown in Tables (19&20). It can be seen that, treated carob cuttings on May following by April were the proper times for giving the highest survival percentage (80.42, 79.46 & 80.56, 79.55 %) in both seasons, respectively, then the value was gradually decreased from June to Sept. in both seasons. Concerning the type of cuttings it is worthy to note that the treated sub terminal cuttings in April was higher than terminal ones (82.02, 81.56) in both seasons respectively. While in May no significant differences between both types of cuttings in both seasons. With regard to growth regulators, the obtained data reveal that, carob cuttings treated with IBA at 9000 ppm + wounding recorded the highest significantly values in both seasons. Concerning the interaction, data clearly showed that, survival % increments were more pronounced with sub terminal cuttings treated with IBA at 9000ppm + wounding in April in both seasons .

These results were in agreement with the findings *of Çelik et al., (1994*) and *Ibrahim et al.,(2009*) on olive.

Plastic tunnels with water mist gave the best rooting and vegetative growth followed by the cutting under plastic tunnels without mist in Hamelia patens, *Elgimabi,(2009)* .

Loach (1997) studied the leaf water potential and the rooting of cuttings of rhododendron under mist and polythene. He stated that, propagation under polythene gave better results than mist in the lower radiation conditions. He studied also the variation in water potential in cuttings under mist and polythene and found that cuttings in mist depended on three variables namely; current days radiation; number of days from insertion of cuttings and either the previous days leaf water potential which was relatively unimportant, probably because cuttings were able to take up water over night from condensation on the under surface of the polyethene.

Table (17): Effect of planting date, type of cutting and treating with hormone on dry weight under tunnel in 2007

Treatments	April		May		June		July		August		Sept.		Mean (A)
	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	4.08	3.96	4.13	4.14	3.92	3.77	3.95	3.73	0.00	0.00	0.00	0.00	2.64
3	4.16	4.16	4.14	4.19	4.28	3.99	5.07	4.50	0.00	0.00	0.00	0.00	2.87
4	4.29	5.35	4.18	4.97	3.92	3.88	4.58	4.00	0.00	0.00	0.00	0.00	2.93
5	4.31	5.21	4.25	4.74	4.05	3.77	4.17	4.37	0.00	0.00	0.00	0.00	2.91
6	4.06	4.95	4.12	5.16	3.58	4.06	3.22	4.75	3.17	3.87	3.00	2.97	3.91
7	4.04	5.18	3.90	5.27	3.18	4.22	4.19	5.08	4.33	4.35	3.19	3.24	4.18
8	4.91	5.55	4.63	5.63	3.84	5.29	4.21	5.07	5.04	5.08	4.97	4.30	4.88
9	5.24	6.14	5.03	6.15	4.59	5.94	5.32	6.03	5.30	4.53	5.25	4.20	5.31
10	5.93	6.09	5.47	6.00	5.03	5.85	4.81	4.70	4.45	4.12	4.03	4.15	5.05
11	6.26	6.83	6.00	6.41	5.50	5.16	5.21	5.16	4.95	4.00	4.89	3.75	5.34
12	6.93	6.96	6.68	6.86	5.82	5.01	5.27	5.11	5.11	4.33	4.83	3.89	5.57
13	7.46	5.42	6.97	4.32	6.49	4.03	6.08	4.22	5.13	4.00	5.09	3.17	5.20
Mean(B)	5.31		5.14		4.55		4.70		2.99		2.70		
Mean(C)	5.14	5.48	4.96	5.32	4.52	4.58	4.67	4.73	3.12	2.86	2.94	2.47	

L.S.D.	A	B	C	ABC
	0.05682	0.04018	0.02320	0.1968

T1= 3000 ppm T2= 3000 ppm + Wounding
T3= 3000 ppm + Wounding + NAA 100ppm T4= 3000 ppm + Wounding + NAA 200ppm
T5= 6000 ppm T6= 6000 ppm + Wounding
T7= 6000 ppm + Wounding + NAA 100ppm T8= 6000 ppm + Wounding + NAA 200ppm
T9= 9000 ppm T10= 9000 ppm + Wounding
T11= 9000 ppm + Wounding + NAA 100ppm T12= 9000 ppm + Wounding + NAA 200ppm

* (A): **Growth regulators** *(B): **Cutting type** *(C): **Planting date**

Table (18): Effect of planting date, type of cutting and treating with hormone on the dry weight (gm.) under tunnel in 2008 season.

Treatments	April		May		June		July		August		Sept.		Mean (A)
	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	Ter.	Sub ter.	
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	4.10	3.83	4.12	4.18	3.93	3.72	3.91	3.76	0.00	0.00	0.00	0.00	2.63
3	4.12	4.24	4.19	4.22	4.28	3.93	5.43	4.59	0.00	0.00	0.00	0.00	2.92
4	4.41	5.32	4.24	5.11	4.05	3.83	4.75	4.18	0.00	0.00	0.00	0.00	2.99
5	4.34	5.24	4.28	4.79	4.18	3.76	4.22	4.21	0.00	0.00	0.00	0.00	2.92
6	4.06	4.86	4.18	5.18	3.75	4.22	3.20	4.82	3.19	3.81	3.00	3.15	3.95
7	3.93	5.22	3.72	5.65	3.23	4.31	4.23	5.13	4.25	4.25	3.15	3.18	4.19
8	4.92	5.65	4.66	6.19	3.82	5.22	4.24	5.22	5.13	5.23	5.22	4.34	4.99
9	5.09	6.10	5.12	6.14	4.70	6.13	5.34	6.18	5.29	4.72	5.16	4.25	5.35
10	5.75	5.59	5.62	6.32	5.17	5.78	4.73	4.59	4.38	4.20	4.22	4.24	5.05
11	6.52	6.75	6.12	6.76	5.69	5.14	5.25	5.14	5.15	4.22	4.83	3.79	5.45
12	7.07	6.68	6.62	7.00	5.81	5.01	5.20	5.19	5.18	4.32	4.94	4.10	5.59
13	7.58	5.33	7.12	4.22	6.58	4.17	6.21	4.26	5.24	4.20	5.15	3.32	5.28
Mean(B)	5.28		5.24		4.60		4.75		3.03		2.75		

Mean(C)	5.16	5.40	5.00	5.48	4.60	4.60	4.73	4.77	3.15	2.91	2.97	2.53

L.S.D.	A	B	C	ABC
	0.0529	0.0374	0.02159	0.1832

T1= 3000 ppm T2= 3000 ppm + Wounding
T3= 3000 ppm + Wounding + NAA 100ppm T4= 3000 ppm + Wounding + NAA 200ppm
T5= 6000 ppm T6= 6000 ppm + Wounding
T7= 6000 ppm + Wounding + NAA 100ppm T8= 6000 ppm + Wounding + NAA 200ppm
T9= 9000 ppm T10= 9000 ppm + Wounding
T11= 9000 ppm + Wounding + NAA 100ppm T12= 9000 ppm + Wounding + NAA 200ppm
* (A): **Growth regulators** *(B): **Cutting type** *(C): **Planting date**

Table (19): Effect of planting date, type of cutting and treating with hormone on the survival (%) after 2 month under tunnel in 2007season.

Treatments	**April**		**May**		**June**		**July**		**August**		**Sept.**		**Mean (A)**
	Ter.	**Sub ter.**	**Ter.**	**Sub ter.**	**Ter.**	**Sub ter.**	**Ter.**	**Sub ter.**	**Ter.**	**Sub ter.**	**Ter.**	**Sub ter.**	
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	77.00	82.13	80.47	80.23	77.27	75.83	0.00	69.30	0.00	0.00	0.00	0.00	45.19
3	75.33	84.20	81.40	81.60	78.47	80.20	71.70	78.43	0.00	0.00	0.00	0.00	52.61
4	75.33	81.87	79.53	80.50	71.77	74.30	70.43	68.13	0.00	0.00	0.00	0.00	50.16
5	72.27	82.90	78.53	78.87	72.50	71.00	70.00	64.57	0.00	0.00	0.00	0.00	49.22
6	73.73	83.57	80.40	81.40	78.63	77.70	72.47	65.03	66.37	66.53	67.37	60.67	72.82
7	75.33	83.57	81.93	80.57	81.40	78.67	74.50	73.37	68.63	70.27	69.10	68.33	75.47
8	78.67	80.17	77.30	80.30	77.23	77.17	70.27	70.50	65.37	64.83	66.27	59.70	72.31
9	70.98	79.50	80.27	78.80	76.40	73.03	67.37	68.73	67.23	63.97	66.47	61.03	71.15
10	81.37	80.70	81.53	81.17	78.80	79.70	74.53	74.50	72.63	77.27	67.00	69.67	76.57
11	82.07	83.53	83.13	82.77	82.43	82.07	75.60	77.67	74.10	78.07	65.17	69.80	78.03
12	80.20	81.77	79.67	80.73	79.13	75.57	74.17	72.87	69.57	70.80	60.27	56.93	73.47
13	80.27	80.57	78.77	80.13	79.37	74.77	71.37	71.67	66.30	67.53	58.87	57.60	72.27
Mean(B)	79.46		80.42		77.23		68.63		46.23		42.68		
Mean(C)	76.88	82.04	80.24	80.59	77.78	76.67	66.03	71.23	45.85	46.61	43.38	41.98	

L.S.D.	A	B	C	ABC
	0.7178	0.5074	0.2929	2.486

T1= 3000 ppm T2=3000 ppm + Wounding
T3= 3000 ppm + Wounding + NAA 100ppm T4=3000 ppm + Wounding + NAA 200ppm
T5= 6000 ppm T6=6000 ppm + Wounding
T7= 6000 ppm + Wounding + NAA 100ppm T8=6000 ppm + Wounding + NAA 200ppm
T9= 9000 ppm T10= 9000 ppm + Wounding
T11= 9000 ppm + Wounding + NAA 100ppm T12= 9000 ppm + Wounding + NAA 200ppm
* (A): **Growth regulators** *(B): **Cutting type** *(C): **Planting date**

From the above mentioned presentation, it could be concluded that, vegetative propagation of Carob by cuttings under mist or under tunnel affected by many factors such as: time of preparing cuttings, type of cuttings, and treated with growth regulators. Rooting ability of Carob, length, dry weight, survival after 2 months and average number of roots increased by increasing concentration of IBA from 3000 ppm to the maximum in 9000 ppm with wounding with NAA at 100 ppm or 200 ppm in both April and May with either terminal or sub-terminal cuttings, but decrease to the little extent in August and September, while fluctuated greatly exhibiting very low values to reach to zero during the period from October to March.

Mist propagation technique costly whereas in areas characterized by poor facilities (electricity and equipments), propagation under white plastic tunnels is cheaper and save the electricity cost, so can be used commercially

Figure (1): Sub-terminal cuttings of Carob after treated with 9000 ppm + wounding under tunnel.

Table (20): Effect of planting dates, types of cutting and treating with hormone on the survival (%) after 2 month under tunnel in 2008 season.

Treatments	April		May		June		July		August		Sept.		Mean (A)
	Ter.	Subter.	Ter.	Subter.	Ter.	Subter.	Ter.	Subter.	Ter.	Subter.	Ter.	Subter.	
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	77.87	82.80	79.90	80.57	76.63	74.83	0.00	70.47	0.00	0.00	0.00	0.00	45.26
3	77.10	84.80	82.27	82.40	78.08	79.40	72.00	79.53	0.00	0.00	0.00	0.00	53.03
4	75.33	81.37	78.73	79.77	70.90	73.64	71.45	69.17	0.00	0.00	0.00	0.00	50.03
5	74.73	81.80	78.83	78.87	71.53	69.53	69.63	65.34	0.00	0.00	0.00	0.00	49.19
6	74.53	82.50	80.67	82.07	79.17	78.03	73.04	65.92	65.50	67.17	65.81	66.13	72.54
7	75.33	84.20	83.07	81.17	81.83	79.41	74.87	75.40	69.63	71.23	68.43	69.33	76.16
8	77.63	79.50	78.17	79.37	77.60	76.27	69.43	71.18	64.62	64.83	66.60	58.37	71.96
9	70.55	79.50	79.40	78.13	75.73	72.43	67.90	69.93	65.64	65.07	67.57	61.87	71.14
10	81.37	82.27	82.17	82.17	80.57	80.83	75.70	75.20	73.34	77.80	67.67	69.37	77.37
11	82.07	84.53	84.13	82.77	83.23	82.77	76.30	79.23	75.03	78.97	65.97	67.77	78.96
12	79.57	80.90	79.83	80.60	79.00	74.73	75.37	71.73	69.03	69.27	61.43	57.63	73.26
13	79.44	79.60	79.20	79.20	79.03	75.77	72.57	70.04	65.50	66.90	59.77	56.60	71.97
Mean(B)	79.55		80.56		77.12		69.26		46.23		42.51		
Mean(C)	77.13	81.98	80.53	80.59	77.78	76.47	66.59	71.93	45.69	46.77	43.60	41.42	
L.S.D.	A	B	C	ABC									
	0.5523	0.3905	0.2255	1.913									

T1= 3000 ppm
T2= 3000 ppm + Wounding
T3= 3000 ppm + Wounding + NAA 100ppm
T4= 3000 ppm + Wounding + NAA 200ppm
T5= 6000 ppm
T6= 6000 ppm + Wounding
T7= 6000 ppm + Wounding + NAA 100ppm
T8= 6000 ppm + Wounding + NAA 200ppm
T9= 9000 ppm
T10= 9000 ppm + Wounding
T11= 9000 ppm + Wounding + NAA 100ppm
T12= 9000 ppm + Wounding + NAA 200ppm

* (A): **Growth regulators** *(B): **Cutting type** *(C): **Planting date**

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Sultanistic regimes, an appropriate model for leaving the problems of bilateral explanations of authoritarianism and totalitarianism

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Abstract: Reviewing the history for the structure of power in the 3rd world expresses the principles of long time existence of non-democratic systems in power. Hence we observe the establishment of Eastern despotic models , Eastern inheriting sultanistic regimes, Asian inheriting despotism and the theories of sultanate systems, expressing this type of structure of power .The theory of sultanistic regimes is a rather modern model , with numerous and different characteristics , than other views , benefiting more strong points and less errors. Therefore , this article is dealing with considering this theory.

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1. Formation of the theory of sultanistic regimes

The concept of sultanistic regimes was created by the conformity analysis of Juan Linz about non-democratic systems. By observing the differences between Franco's regime and Trujillo's regime due to visiting an exiled Spaniard called Jesus De Galindez, who had analyzed the secret performances of Trujillo's regime, he expressed a type of political system and selected the expression of "Weber sultanate system", for it.(J. Linz, and H. Chehabi , 1380:15-16)

The most important characteristics of Trujillo's system, in inspiring the primary formulation of the concept of sultanistic regimes , include: government individualism, loyalty due to apprehension and rewards , intervention in economy, widespread corruption, centralism, formation of private army, combination of public and private roles, developing military relations with the U.S.A , presence of sovereignty crisis, obstinate decision making and maintaining legal out looks, establishing the titles of "his highness", Dr. Trujillo", "Honorable president", "Supporter of the nation", "Provider of the country's financial independence", etc,… But, his seeming obligation for discipline, establishing an integrated national government and economic nationalism led to the general admission of his nation. (j. Linz,1970: 255-90)

Linz believes that:" The differences between sultanistic regimes and totalitarianism is not only referred to the rat of difference, but generally politics from the view point of the relevant rulers to the structure of power related to social and economic structures and the related people to such sovereignty." (j.j.Linz, 1975)

He believes that in contrast to sultanistic regimes, totalitarian systems are expressed as a real ideology, by the enlightened people supporting the system, to provide legitimacy and establish the policies, and the leaders and their followers believe in their prophecy and show a mortifying view of themselves. The possibility of gathering policies is provided by the party, subordinate organizations, such as the youth organizations, women's organ, etc. and the base for promoting partnership in the community is established and influential aspects are fulfilled in different fields of the community in quite a harmonious manner. (j.Linz, and H.Chehabi, 1380:57-60)

The most important differences of authoritarian and sultanistic regimes include: Due to existence of limited social and political pluralism in authoritarian systems, the opportunity of selecting the elite is provided from among different structures, but the arbitrary method by the ruler in sultanistic regimes in selecting the assistants, lack of predictable ways for occupational progress and trivial supportive structures is in contradiction with authoritarian systems. (j.Linz, and H.Chehabi, 1380: 60-1)

Distinctions between the people having political authorities and the individuals performing actions related to the modern government, in authoritarian systems, is possible by politicizing the community, but the ambiguity for the boundaries between the system and the government is evident in sultanistic regimes

2. Sultanistic regimes according to Max Weber and other:

According To Weber: "Each dominant tries to arise legitimacy for himself, among the followers". (J. Freund, 1362:240)

In this way, he defined the word "legitimacy" for the first time (A. Nabavi , 1379 :448) and recognized 3 types of legitimate domination.

In moral political system, which is known as" the most impersonal type of domination"(J. Freund, 1362:240), power is due to a set of organized regulations, related to positions and based on individual merits. Weber has referred to Rudolph Sohm (M. Weber,1382:62) for the word "Charisma", that is the specific characteristic of a person separated from ordinary people due to these characteristics, and uses this word for a person that his capabilities or ultra natural, extraordinary and exclusive character. (M. Weber, 1374:397) Hence the center of such system is the charismatic leader that obtains his legitimacy by the admission of public. (A .Bakhshayeshi, 1376:97)

According to Weber, the oldest type of legitimacy is based on the sanctity of tradition (M. Weber, 1368: 100, M. Weber, 1374: 42), I.E. the domination of the traditions that "have got sanctity from the old ages, due to acceptance and admissions". (H.Gerth and C.Wright Mills, 1970:78-90) According to Weber the traditional domain has a bilateral field: "The holy tradition and the ruler's authorities". According to Weber; "Patrimonialism is" a form of traditional political sovereignty that a royal family performs cruel power by a judicial establishment".(A.Ashraf ,1347:12)Ahmad Ashraf recognizes the basic for creation of Patriomonialism by the Weber's point of view as the construction of power known as royal father, that means if the political system lacks administrative officers, it will be called Patriarchy and if the system has administrative organization, it will be called patrimonial or Feodal. (A.Ashraf, 1347:12)

Like Ashraf, Turner believes: "By expansion of the roles and judicial duties, the patrimonial ruler has to assign a large no of workers as slaves inside its judicial system. It is why that patrimony is transformed to inheriting domain. (S.Hajariyan, NO.91-92:45-57)

Kirth and Mills point to a second basis, which is establishing the royal specific rights, which is based on the respect of the sovereign power. (S.Hajariyan, NO.91-92: 46)

The most important specifications of Patrimonilism include: Being close to bureaucratic that lacks mental and technical element and is the private tool of the ruler, and the uncontested parade of the ruler in economic and social field without any responsibilities for maintaining boundaries and classic obstacles, intermediately capital growth and fear from faithful factors. In addition to introducing a specific type of Patrimonialism, Weber believes that: "In case of extraordinary power of the master, Patrimonialism tends to sultanate, Friends are transformed to peasants, the master's right that up to them was considered the head of the followers, is transformed to the personal right of the master and if the patrimonial supremacy relies on the authority and autocracy without observing the tradition, it is called the royal supremacy." (A.Azghandi, 1384:27-9. M. Weber, 1374 :329)

It is also said that: "Apparently the royal form of Patrimonialism is sometimes completely dependent on tradition, but it is never in real estate."(M. Weber, 1374:329)

Hence we observe a paradox from one side, Weber place the traditional basis of Patrimonialism in unfrontation with the personal authority in sultanistic regimes and on the on the other side recognizes monarchies as totally dependent on tradition. Goodin and scotchpoul believe: "A new patrimonial and sultanistic engine is a system that the power is in the hands of a dictator ruler that never allows the deployment of any groups with political stabilities in a political atmosphere"(S.Hajariyan, NO.91-92: 48)

Scholochter recognizes" The sultanistic regimes as a kind of extremist Patrimonilism, in which the authorities of the king is overrated.(M. Weber and others,1379:10)

Turner believes": Whenever the patrimonial authority could be released from tradition limits, it is called sultanate"(S.Hajariyan, NO.91-92: 48)

Roth also knows "The personal sovereignty as a type of de-traditionalized Patrimonialism".(P. Broker,1383,P.92)

Hajarian believes that "according to Linz, if a patrimonial government is restored trivially it gradually gets closer to sultanate ". (S.Hajariyan, NO.91-92: 48)

But Linz and Chehabi believe: "A system with some sultanistic regimes inclination that has expanded loop of followers, but the freedom and authority of the ruler is less, should be named as new Patrimonialsm and when this loop is getting tighter, this system me be transformed to sultanate system". (j. Linz, and H.Chehabi1380: 27-8, 18)

As defined, some researchers according to Hajarian namely Weber, Turner, Scholocher ,Routh and Linz -recognize the extreme state of Patrimonialism as sultanate system and some other including Goodin, and scotchpaul believe in the similarity of new Patrimonialism and sultanate, while some others including Linz and Chehabi recognize the new patriomonial extremism as sultanistic regimes .

3. Sultanistic regime by Juan Linz and Houshang Chehabi theory:

Some characteristics of the idealist type of contemporary sultanistic regimes from the view points of Linz and Chehabi:

Faithful motivations as a combination of fear and awards, (j.Linz, and H.Chehabi1380: 21)

Applying the ruler's power arbitrarily, with no legal abstraction or ideological obligations, weakening the obligating manners by personal decisions, Promoting corruption, selection of administrative and military personal by the ruler-independence of the ruler and his followers from any specific group-weakened legitimacy and lack of ideological justifications. In addition to the above points, Linz and Chehabi recognize 5 common characteristics of sultanistic regimes as: Tension regarding the boundaries between the system and the government, individualism, legal hypocrisy, limited social places, altered capitalism.

Tension regarding the boundaries between the system and the government:

system, with the meaning of gathering and combing(A.Biro, 1375:419) is a set of non-materialist elements depending in each other that by combination of the elements an organized combination is created and "the relevant commands are fulfilled in defined geographical limit".(A.Ghavam,1374:64.A.Alam,1373:150)Linz and Chehabi defines the system as "allocated models of power, using and misusing power in government "and recognize its inclusions as something more than the constitution of power in a democratic system and less than expanded structures of dominations in totalitarian system(j. Linz, and H.Chehabi,1380:28)

and express 3 fields of bureaucratic structures, armed forces organization and parties as the sub-systems.

Bureaucratic structures:

The most important characteristics of it include: Direct intervention of the ruler and the assistants in the structures and not considering the vocational standards ,lack or distorting moral norms ,apparent existence of modern bureaucracy, entering the technocrats in to the system with the aim to please Americans supporters ,replacing non-political and non-authorized aspects for political disorders, agreeing with somewhat rationalizing affairs for increasing the capability of resource exploitations, loyalty of an official is the loyalty of a servant based on a precisely personal relation to the ruler(j. Linz, and H.Chehabi,1380:32) lack of employment security.

Armed forces organization:

In this field, we observe: Ruler's actions to extend mistrust among different branches of army providing spies among the armed forces personal, establishing private militias, lack of unique commands, lack or distorting of promoting criteriones. In this way, the armed forces leave their professional condition.

(j. Linz, and H.Chehabi, 1380:32)

The party organization:

In such systems, we observe a single party system by the ruler. The name of the party could indicate "The efforts of the ruler to show the system, as for the revolutionary movement leader". (j. Linz, and H.Chehabi, 1380:35) Thus the party does not follow the normal trends and lacks legitimacy, Political selections and arranging the strategies.

Individualism:

It means that the person benefits from a complex of social and individual qualities and the community provides the individuality and possession of power.

Individualism has the two aspects of personality faith and inclination towards family orientation. (j. Linz, and H.Chehabi, 1380:35)

Personality faith:

includes the following :These leaders lack the wise leadership arrays and therefore tend towards establishing new titles for themselves and like to be considered as great intellectuals, Thus lots of books are filled by their notes and lectures and speeches and many books are published in their names and the selective ideology should be: legitimizing and reflecting of individualism and consists of the name of the ruler and benefits an inventing tradition to distinguish the nation from the neighborhood. More over" whatever the sultanate aspects are more pure, it will be more possible that the ideology is formed to justify the rulers. (j. Linz, and H.Chehabi, 1380: 37-8) the leader likes to have his name and picture and his interesting objects to gain the required authority in all over the country.

Inclination towards family orientation is due to the lack of social tribal, social economic trusting supports of monarchs. Humiliating looks of the elite to these leaders force them in addition to getting rid of some of the elite people, to gain the cooperation of a part of them by providing family unities and homogeneities with old elite and outstanding people.

Legal hypocrisy means that the leader seemingly respects the laws and "benefits from any opportunities to enlight democracy, although he presents some other concepts for it"

(j. Linz, and H.Chehabi,1380: 45) They are in the place That the governmental responsibilities are undertaken by others, intervention in elections, violating civil liberties of different groups , referring to public votes by the aim of representing democratic

society and proving legitimacy and the views of a charismatic leader are among the other indications in this regard.

Limited social base:

Social aspect is a condition that anyone obtains in social structure by the merit of social evaluations. (A.Nikgohar, 1375:64.B.Sarukhani, 1370:729. A. Biro, 1375:380-1)

Linz and Chehabi believe that the leaders of sultanistic regimes get into the power by the support of quite specific groups, but they do not attempt in maintaining the primary supports and deliberately try to omit the supports, since the individual -oriented nature of such governments requires the sense of not needing the coalition with the existing groups in the civil community. Therefore: "They lose their primary social supports and rely on fear and awards".(j. Linz, and H.Chehabi,1380:49)But the ruler provides supports from two domestic groups, family members & friends and the residence in the place of birth and a foreign group; mainly U.S.A with regards to his personal authorities. (j. Linz, and H.Chehabi, 1380:51)

Distorted capitalism:

The first point of capitalism is the primary savings, (Chilkut, 1375:13) but the sultanistic regimes "Prevents savings of the capital "by weakening responsibilities. (j. Linz, and H.Chehabi, 1380:56)

Thus, we observe the individualistic use of power for the purposes that are mainly personal for the ruler and his followers, concluding generous contracts between the ruler's economic establishments and the government, exclusive ownership of vital materials, ignoring contracts, evident confiscation of people's properties and assets, and the dependent economy for intervening the government is through controlling principle servicing. Wise economic activities are also for the aim of maximizing economic profit abilities" (j. Linz, and H.Chehabi, 1380:56)

4. The reasons of formation of sultanistic regimes according to Linz and Chehabi:

Linz and Chehabi point to 3 factors, in the reasons for creation of these systems: macro structures-social_ economic conditions & international atmosphere (ruling crisis), fundamental political factors & leadership.

Marco-structures:

From the view point of Linz and Chehabi, stability of sultanate system requires renovation of transport systems, military organizations and some offices to supply financial sources. Seclusion of rural population and lack and/or poor education is necessary for their effective obedience. (j. Linz, and H.Chehabi, 1380: 64) Therefore, the restoration of the 3 fields of transportation and communication, military organization, bureaucracy and sociology analysis of the rural areas are emphasized. Contrary to Weber, they believe in the importance of the economic factor. They believe: the financial resources of the governments in small countries could be expanded via customs charges and in more complex communities, the financial resources are provided by the parts not needing work force in large industries, clients, modern administration systems, civilization, expanding of education, and the natural resources with easy exploitations and high profits that belong to one or more institutions are considered as proper financial and income sources. (j. Linz, and H.Chehabi, 1380:65) Also "the foreign aids or loans (in case of having no peculiar conditions) could encourage corruption". (j. Linz, and H.Chehabi, 1380: 66)

Sovereignty is the supreme power to issue commands. Linz and Chehabi believe that the "independence of such countries are somewhat vague and they are not respected by more powerful neighborhoods." (j. Linz, and H.Chehabi,1380:68)

They believe that" The internal crisis and instabilities provide the conditions primarily that facilitates the interventions of foreign powers and then the foreign interventions lead to creation of systems that replace internal collations for foreign supports and could lead to sultanistic regimes. Therefore the sovereignty crisis originated from domestic conditions ends up in increasing the crisis in the community macro-structure and by revealing the in efficiency of the existing structures, the mentality would be provided for the necessity in changes in the structures by an efficient and strong government.

Fundamental political factors:

An organization or a foundation is a sub-system of official groups (P. Meyer, 1375: 126) and "a type of human relations model forming materialistic and realistic relations "(M.Dovorje, 1376:118), with the aim to provide human requirements. Hence, the necessity for organizing constitutions is to be considered. Linz and Chehabi believe that " sultanistic regimes do not have specific constitution. (j. Linz, and H.Chehabi, 1380:78) Of course they mean that in such systems, the establishments are deviated from their own special tasks and are totally placed under the considered plans of the ruler.

Thus the ruler's dominations influence the establishments. They believe the supporting democracy collapse and dissolution of non-democratic systems leads to sultanistic regimes. The most important characteristics of supportive democracy include: Existence of multi-party system and entering or leaving the numerous parties to or from politics and facilitating the process for the

involvement of the elite. Thus the privatization of power structure would reduce. They believe that by the collapse of supportive democracy. The no. of supporters would reduce to only one supporter". (j. Linz, and H.Chehabi,1380:78) Such that: The possibility of parties (non-governmental parties) is provided by replacement of supportive democracy, in addition to maintaining the appearance of the former government, but the permanent dominance of a governmental party leads to dissolution of constitutions, monopolization of resources in the hands of the ruling party and individualism, and also the lack of obligation of the parties in these systems with regards to the party's ideologies could lead to concentration of supportive policies based on personal dependencies, dissolution of establishments and damaging legal systems against changes of parties variability and manipulations from top levels. Widespread corruption of democratic governments and destruction of the possibility for involvement of the elite are among the other indications."The public interests in authoritarianism systems are defined by the governments and involvement of people in politics is viewed as a suspecting phenomenon or abolishing phenomenon". (E.Tansi, 1376:162-3)

Linz and Chehabi believe that the traditional authoritative regimes (dictator regime) like china prior to the modern era "dealt with showing of their power after the death of the emperor and dependent servants". (j.Linz, and H.Chehabi1380:80)

Hence, changing the deciding mechanisms indicate the dissolutions. "The seniority principle in the west too used to avoid the appearance and formation of competitive royal groups". (j.Linz and H.Chehabi1380:80)

The modern authoritarian systems, such as the second period of Batista is Cuba and Duvallier government in Haiti became non-constitutive and the rulers became more corrupt, and the lack of considering constitutional laws in the country and ignoring the maintenance of the supportive network increase. Dissolution of totalitarian regimes such as the 1968 coup of Ba'ath party is defined by the presence of the king's family and relative in governmental organizations and their intervention in different fields.

The leadership factors:

In the "Twelfth night" play, Shakes pear wrote: "Some became aristocrats, some achieve aristocracy and aristocracy is imposed to some others".(D.Apter,1380:416)

Deep considerations of these phrases indicate that obtaining the leadership position depends on elements such as mutual actions between personal characteristics and social – political conditions. According to Linz and Chehabi, the founders of sultanistic regimes have limited education, arose from peasant families, their ascending movement is usually occurred by accident, they are clever, they are morally untruthful and revengeful, they have the ability to lie and are adulterous ,they are hedonists and often show personal ruthlessness(j. Linz, and H.Chehabi,1380:87-8)alongside these factors, they consider the element of "accidental" aspects, (j. Linz, and H.Chehabi,1380:87) like Machiavelli.

Therefore they have focused on 3 fields: family structure characteristics, individual and mental characteristics, and the role of the accident in hegemonism.

5. Conclusion:

The positive points of the theory include: simultaneous considerations to micro-elements (role of the leader) and macro-elements (economical-social-constitutional and international) ,simultaneous considerations to the structure and functional factors , avoiding the inclination towards determinism, combination of the elements of the theory from different theories such as Asian production method, John Fouran, shapour Ravasani, Huntington, Fred Holiday, etc-generalizing this model to all the countries that have not moved for social and economic evolutions.

Therefore a combination of internal and external factors determine the form and nature of the governments in the communities and determine the government oriented approach of this theory and direct consideration of the pathology of the modern government and inclination towards relativism that this of course could be an open rout for an evident escape and some in evident run-away to escape from criticisms of the critics.

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Synthesis and Biological evaluation of pyrrolo[2, 3-*d*]pyrimidine derivatives as antibacterial and antiviral

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Abstract: A new class of pyrrolo[2,3-*d*] pyrimidine derivatives has been designed and synthesized, then examined and evaluated for their antiviral and antibacterial activity. New prophylactic therapeutic tools are needed for the treatment of highly pathogenic avian influenza (HPAI) H5N1 and highly virulent Newcastle disease virus (NDV). **3a, 3b, 3g, 3h** have shown to posses highly potent against highly pathogenic avian influenza (HPAI) H5N1 virus and **1a, 2a** are shown highly potent against highly virulent Newcastle disease virus (NDV),and the compounds **1a-c , 2a, 2b, 3a, 3g, 3h** are shown highly potent against enterobacteriases [*Escherichia coli* and *Salmonella typhimurium*] strains. This study is the first record in Egypt and may be in the world concerning the activity of these new class of pyrrolo[2,3-*d*]pyrimidine derivatives against (HPAI) H5N1 and NDV.
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Key words: Pyrrolo[2, 3-*d*]pyrimidine derivatives , antibacterial , antiviral activity

1. Introduction:

Compounds containing pyrrolopyrimidine functional groups, collectively referred to as 7-deazapurines, are a structurally diverse class of nucleoside analogs with demonstrated antibiotic [1]. These compounds have been intensively investigated as antitumor [2,3], Cytotoxicity[4] anti-allergic[5], antiviral[6], antimicrobial and anti-inflammatory agents [7].

During the past ten years a series of avian epidemics, significant reemergence of highly pathogenic avian influenza (HPAI H5N1) has been reported in several Asian, countries including Hong Kong, Korea, Japan, Taipei, Cambodia, China, Indonesia and Thailand, with additionally confirmed cases of human death in Vietnam [8].

With continued outbreaks of the H5N1 virus in poultry and wild birds, further human cases are likely, and the potential for the emergence of a human adapted H5N1 virus either by reassortment or mutation, is a threat of public health worldwide. Limitation in our current HPAI treatment options and the continuing out breaks of the H5N1 viruses have contributed to a growing need for new and effective chemotherapeutic agents to treat viral diseases.

Also New Castle disease is a highly contagious, septicaemic, fatal and destructive disease which attack chiefly chickens and turkeys usually in an acute, sometimes in sub acute or even chronic form. Occasionally human being and even wild birds may be also infected with the virus. New castle disease (ND) caused by virulent virus, is one of the most infectious diseases of poultry and induces up to 100% mortality as well as a decrease in egg production and poor egg quality [9]. ND is regarded throughout the world as one of the most important diseases of poultry, not only due to the serious disease and high flock mortality that may result from some ND virus (NDV) infections, but also through the economic impact that may ensue due to trading restrictions and embargoes placed on areas and countries where outbreaks have occurred [10].

On the bases of these observations, we report the synthesis of a novel series of pyrrolo[2,3-*d*] pyrimidine derivatives and evaluate them as antiviral and antibacterial.

2. Materials and Methods

Biological activity

Pyrroles **1a-c** and Pyrrolo[2,3-*d*]pyrimidine derivatives **2a-c, 3a-i** synthesized as described before, stock solutions of all compounds were prepared at 10 mg /ml in dimethyl sulfoxide then make different concentration.

Highly pathogenic avian influenza (HPAI) H5N1 virus A/chicken/Egypt/9402 NAMRU3-CLEVB 213/2007 (H5N1) of accession No.EU 623467 obtained from Central laboratory for Evaluation of Veterinary Biologics (CLEVB), was used at titer of 106 EID 50/ml (Embryo infective dose fifty per ml).

New Castel Disease Virus (NDV) local virulent strain obtained from viral strain bank of Central Laboratory for Evaluation of Veterinary Biologics (CLEVB), NDV was propagated by using SPF chicken eggs, via allantoic sac route and stored at -70°C until used its titer was 10^6 of EID_{50} /ml Growth medium, MEM (minimum essential medium) produced by (Gibco), Was supplemented with 10% new porn calf serum at pH about 7.2 for primary and secondary cultures. Trypsin (1.250) produced by (Gibco), used in a concentration of 0.25% as a cell dispersing agent [11]. Cell culture of African green monkey kidney cells(Vero cell)[12].

Specific pathogen free (SPF) embryonated chicken eggs (ECEs) were used at nine days old and inoculated via allantoic sac route.

Escherichia coli and Salmonella typhimurium obtained from bacterial strain bank of Central Laboratory for Evaluation of Veterinary Biologics (CLEVB).

Mueller-Hinton broth (oxide), this medium was used in the propagation of the bacterial culture. Mueller-Hinton agar[13], this medium was used in the disc diffusion test .it produces a large and clear zone of inhibition when sensitive organisms come in contact with the susceptible antimicrobials compounds.

Toxicity using embryonating chicken eggs (ECEs)

Five hundred and twenty five SPF embryonating chicken eggs (ECEs) of 9 days old were used as following:

Each compound have 6 dilution (ten-fold dilution) and in each dilution we use 5 (ECEs) inoculate with 0.2 ml/ECE via allantoic sac and incubate all eggs for 5 days; through each all eggs candled every day.

Cytotoxicity assays

Various concentration (6 dilution of each compounds) were added to confluent CEF cell and

Vero cell monolayer cultures, 24hr after seeding are maintained for 4 days then viable cell counts of each culture were determined daily by inverted microscopic inspection of cells that`s not affected by compounds .

Antiviral activity

Using SPF embryonating chicken eggs (ECEs) as described in exp. (1)

Equal volume of New castle disease Virus (NDV) was mixed with equal volume of non toxic concentration of each compound and incubated for one hour at room temperature then inoculated into allantoic sac of five ECEs for each concentration of each compound at a dose of 0.2 ml/ECE.

Also in each compound there are 5 ECEs were inoculated with the (NDV) that mixed with equal volume of saline at a dose 0.2 ml/ECE (positive control), also another 5 ECEs were inoculated with 0.2 ml/ECEs of saline alone (negative control), then all the ECEs were incubated at 37 ^{0}C and candled every two hours till all positive controls ECEs were died.

All the embryos of the positive controls were died and allantoic fluid of each was positive for haemagglutination assay (HA), while all the embryos of negative controls were not died and allantoic fluid of each was negative for HA.

Haemagglutination activity of the allantoic fluids of inoculated eggs is measured by micro technique of Haemagglutination test[14].

The previous mentioned steps were repeated with HPIA (H5N1)

Antibacterial activity

Finally, compounds **(1a-c), (2a-c)** and **(3a-i)** were screened for antibacterial activity using disc diffusion method[15].

The microorganisms used in this study were Escherichia coli, sallamonella typhimurium (gram-negative bacteria), all compounds of non toxic concentration of were dissolved in DMSO (100 µg/ml) and 25 µl of them were loaded to 6mm paper discs, 100 µl of 10^9 cell /ml suspension of the micro organisms were spread on sterile Muller-Hinton agar plates and the discs were placed on the surface of culture plates, then incubate for twenty four hour to determine zone of inhibition as shown in Table (3).

Antiviral Effect of Compounds **1a-c, 2a-c** on NDV **(Original virus titer EID_{50} /ml = 10^6)**

Compound	R	R_1	(MIC) µgm/ml	NDE	+ HA	Final EID_{50} of virus
1a	H	H	2.6	0/5	0	0
1b	4-Cl	H	3.2	0/5	0	0
1c	4-Br	H	3.4	0/5	0	0
2a	H	H	2.7	0/5	0	0
2b	4-Cl	H	3.2	2/5	2	10^2
2c	4-Br	H	3.6	0/5	0	0
Ribavirin			3	0/5	0	0

MIC: minimum inhibition concentration.
NDE: no. of died eggs,
+HA: positive haemagglutination.
EID_{50}: (Embryo infective dose fifty per ml).

Table 2. Antiviral Effect of Compounds **3a-i** on H5N1 (Original virus titer EID_{50} /ml=10^6)

compound	(MIC) µgm/ml	NDE	+HA	Final EID_{50} of virus
3a	3.8	0/5	0	0
3b	3.6	1/5	1	10
3c	41	0/5	0	0
3d	40	2/5	2	10^2
3g	4.5	0/5	0	0
3h	4.1	0/5	0	0
3i	49	0/5	0	0
Ribavirin	6	1/5	1	10

MIC: minimum inhibition concentration. NDE: no. of died eggs.
+HA: positive haemagglutination. EID_{50}: (Embryo infective dose fifty per ml).

Table 3. Antibacterial Activity of compounds 1a-c, 2a-c, and 3a-i

Zone of inhibition(mm)					
Compound	**R**	**R_1**	**R_2**	**Escherichia coli**	**Salmonella Typhimurium**
1a	H	H		19	18
1b	4-Cl	H		17	15
1c	4-Br	H		18	17
2a	H	H		19	16
2b	4-Cl	H		19	18
2c	4-Br	H		15	14
3a	H	H	$-CH_2CH_2OCH_2CH_2OH$	14	12
3b	H	H	$-CH_2CHOHCH_2OH$	14	12
3c	H	H	$-(CH_2)_8CH_3$	9	8
3d	4-Cl	H	$-CH_2CH_2OCH_2CH_2OH$	8	8
3e	4-Cl	H	$-CH_2CHOHCH_2OH$	9	8
3f	4-Cl	H	$-(CH_2)_8CH_3$	7	7
3g	4-Br	H	$-CH_2CH_2OCH_2CH_2OH$	14	12
3h	4-Br	H	$-CH_2CHOHCH_2OH$	14	13
3i	4-Br	H	$-(CH_2)_8CH_3$	9	10
Sutrim				13	12

Table 4. MIC (µg/ml) Value of Compounds 1a-c, 2a, 2c & 3a, 3b, 3g, 3h on Escherichia Coli and Salmonella Typhimurium

Comp	R	R_1	R_2	Escherichia coli (µg/ml)	Salmonella typhimurium (µg/ml)
1a	H	H		2.6	2.6
1b	4-Cl	H		2.9	2.9
1c	4-Br	H		3.4	3.4
2a	H	H		2.8	2.8
2b	4-Cl	H		3.2	3.2
2c	4-Br	H		3.6	3.6
3a	H	H	$-CH_2CH_2OCH_2CH_2OH$	3.7	3.7
3b	H	H	$-CH_2CHOHCH_2OH$	3.6	3.6
3g	4-Br	H	$-CH_2CH_2OCH_2CH_2OH$	4.5	4.5
3h	4-Br	H	$-CH_2CHOHCH_2OH$	4.4	4.4
Sutrim				< 4	< 4

Experimental

Chemistry

Melting points were measured with a Gallenkamp apparatus (Weiss-Gallenkamp, London, UK) and are uncorrected. The IR spectra were recorded on KBr pellets on a Jasco FT/IR 460 plus (Japan). ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Gemini 200 MHz in DMSO-d_6 or $CDCl_3$ as solvent, using tetramethyl-silane (TMS) as internal reference standard. The chemical shifts values are expressed in ppm (parts per million). Elemental analyses were performed by a Vario III CHN analyzer (Germany). All compounds were within ± 0.4% of the theoretical values. Mass spectra were run on DI analysis Shimadzu QP-2010 plus mass spectrometer. All spectroscopic data and elemental analysis were made at the Microanalytical Unit of Cairo University. The progress of the reaction and purity of the compounds were monitored by TLC analytical silica gel plates 60 F_{254}. The chemical reagents used in synthesis were purchased from Fluka, Sigma and Alderish.

General procedure for the synthesis of compounds 1a-c

Derivatives of phenacylmalononitrile were reacted with aniline in the presence of ethanol and conc HCl under reflux to give pyrrole derivatives 1a-c [16].

General procedure for the synthesis of compounds 2a-c

A mixture of 1,5-disubstituted-2-amino-3-cyano-1*H*-pyrrole (1a-c) (0.015mole) in formic acid (20 ml) was refluxed for 4 h. Then the reaction mixture was cooled and the separated solid was filtered, washed with ethanol, dried and crystallized from ethanol.

6,7-Diphenyl-3,7-dihydro-pyrrolo[2,3-*d*] pyrimidin-4-one 2a

Yield: 48%; mp: 292-293 ^{o}C; IR ν_{max} [cm^{-1}]: 3108 (NH), 1680 (C=O), 1596, 1484 (C=C, C=N); ^{1}H NMR (DMSO-d_6) δ ppm: 6.83 (s, 1H, $H_{pyrrole}$), 7.23-7.88 (m, 10H, $H_{arom.}$), 8.10 (s, 1H, $H_{pyrimidine}$), 10.1 (s, 1H, NH); ^{13}C NMR δ ppm: 104.31, 124.62, 128.01, 130.53, 135.39, 139.12, 140.11, 143.41, 147.73, 149.35, 157.69 (aromatic carbons) and 176.34 (C=O); Anal. Calcd for $C_{18}H_{13}N_3O$ (287): C, 75.26; H, 4.52; N, 14.63. Found: C, 75.50; H, 4.43; N, 14.67; MS m/z [%]: 287 [M^+] (100).

6-(4-Chlorophenyl)-7-phenyl-3,7-dihydro-pyrrolo [2,3-*d*]pyrimidin-4-one 2b

Yield: 87%; mp: 320-321 ^{o}C; IR ν_{max} [cm^{-1}]: 3427 (NH), 1668 (C=O), 1588, 1484 (C=C, C=N); ^{1}H NMR ($CDCL_3$) δ ppm: 6.88 (s, 1H, $H_{pyrrole}$), 7.23-7.85 (m, 9H, $H_{arom.}$), 8.13 (s, 1H, $H_{pyrimidine}$), 11.7 (s, 1H, NH); Anal. Calcd for $C_{18}H_{12}N_3ClO$ (321): C, 67.29; H, 3.74; N, 13.1. Found: C, 67.30; H, 3.5; N, 13.2; MS m/z [%]: 321 [M^+] (100).

6-(4-Bromophenyl)-7-phenyl-3,7-dihydro-pyrrolo [2,3-*d*]pyrimidin-4-one 2c

Yield: 84%; mp: 340-341 ^{o}C; IR ν_{max} [cm^{-1}]: 3427 (NH), 1668 (C=O), 1588, 1484 (C=C, C=N); ^{1}H NMR ($CDCL_3$) δ ppm: 6.86 (s, 1H, $H_{pyrrole}$), 7.26-7.87 (m, 9H, $H_{arom.}$), 8.13 (s, 1H, $H_{pyrimidine}$), 11.6 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{18}H_{12}N_3OBr$ (367): C, 58.86; H, 3.27; N, 11.44. Found: C, 59.23; H, 3.5; N, 11.5; MS m/z [%]: 367 [M^+] (100).

General procedure for the synthesis of 3,6-substituent of Pyrrolo[2,3-*d*]pyrimidin-4-ones 3a-i.

Compounds 2a-c (0.006 mol) of each was suspended in dry DMF (30 ml). To this suspension was added sodium hydride (60%, 0.24 g, 0.006 mol). The mixture was stirred at room temperature for 20 minutes and then the alkyl halide (0.006 mol) was added. The reaction mixture was refluxed for 5 hours. Then the mixture cooled to room temperature and the solvent was evaporated under vacuum. The residue was dissolved in dichloromethane (200 ml), and the organic phase was washed with water (2 x 50 ml). The organic layer was dried over sodium sulfate and evaporated. The residue was purified by chromatography on silica gel column (2 x15 cm) and the product was eluted with $C_2H_5COOCH_3$ / $CHCl_3$.

3-{2-(2-Hydroxyethoxy)ethyl}-6,7-diphenyl-3H-pyrrolo[2,3-*d*]pyrimidin-4(7H)-one 3a

Yield (75%); m.p.280-281^{0}C; IR (KBr) ν_{max} [cm^{-1}]: 1678 (CO), 3419(OH); ^{1}H NMR (DMSO) δ ppm: 2.34 (s, 1H, OH), 3.09-3.15 (t, 2H, N-CH_2), 3.48-3.56 (d, 2H, O-CH_2CH_2OH), 3.63-3.71 (d, 2H, N-CH_2CH_2),3.76-3.82 (t, 2H, CH_2OH) 6.83 (s, 1H, $H_{pyrrole}$), 7.23-7.88 (m, 10H, $H_{arom.}$), 8.10 (s, 1H, $H_{pyrimidine}$); MS m/z [%]: 374.95 [M^+] (12). Anal.Calcd. for $C_{22}H_{21}N_3O_3$ (375.42): C, 70.38; H, 5.64; N, 11.19.Found: C, 70.45; H, 5.25; N, 11.05.

3-(2,3-Dihydroxypropyl)-6,7-diphenyl-3H-pyrrolo[2,3-*d*]pyrimidin-4(7H)-one 3b

Yield (75%); m.p.283-285^{0}C; IR (KBr) ν_{max} [cm^{-1}]:1686 (CO), 3403(OH); ^{1}H NMR (DMSO) δ ppm: 2.49 (s, 1H, OH), 2.70-2.72 (d, 2H, N-CH_2), 2.87-2.88 (d, 2H, OH-CH_2), 3.25-3.39 (m, 1H, CH), 6.89 (s, 1H, $H_{pyrrole}$), 7.15-7.89(m, 10H, $H_{arom.}$), 8.11 (s, 1H, $H_{pyrimidine}$); MS m/z [%]: 361.05[M^+] (7). Anal.Calcd. for $C_{21}H_{19}N_3O_3$ (361.39): C, 69.79; H, 5.30; N, 11.63.Found: C, 70.45; H, 5.25; N, 11.35.

3-Nonyl-6,7-diphenyl-3H-pyrrolo[2,3 *d*]pyrimidin-4(7H)-one 3c

Yield (65%); m.p.125-127^{0}C; IR (KBr) ν_{max} [cm^{-1}]:1684 (CO); ^{1}H NMR (DMSO) δ ppm: 0.71-0.89 (t, 3H, CH_3), 1.20–1.67 (m, 10H, $5CH_2$), 2.45-2.59 (m, 2H, CH_2), 3.19-3.43 (t, 2H, N-CH_2), 6.85 (s, 1H, $H_{pyrrole}$), 7.11-7.57 (m, 10H, $H_{arom.}$), 8.21 (s, 1H, $H_{pyrimidine}$); MS m/z [%]: 413 [M$^+$] (100). Anal.Calcd. for $C_{27}H_{31}N_3$ O (413.55): C, 78.42; H, 7.56; N, 10.16.Found: C, 78.75; H, 7.25; N, 10.35.

3-{2-(2-Hydroxyethoxy)ethyl}-6-(4-chlorophenyl)-7-phenyl-3H-pyrrolo[2,3-*d*] pyrimidin-4(7H)-one 3d

Yield (64%); m.p.278-279^{0}C; IR (KBr) ν_{max} [cm^{-1}]:1680 (CO), 3422 (OH);^{1}H NMR (DMSO) δ ppm: 2.39 (s, 1H, OH), 3.08-3.19 (t, 2H, N-CH_2), 3.43-3.57 (d, 2H, O-CH_2CH_2OH), 3.61-3.70 (d, 2H, N-CH_2CH_2), 3.77-3.84(t, 2H, CH_2OH), 6.90 (s, 1H, $H_{pyrrole}$), 7.25-7.85 (m, 9H, $H_{arom.}$), 8.14 (s, 1H, $H_{pyrimidine}$); MS m/z [%]: 409 [M$^+$] (7.30),411 [M^{+2}] (5). Anal.Calcd. for $C_{22}H_{20}ClN_3O_3$ (409.78): C, 64.47; H, 4.92; Cl, 8.65; N, 10.25.Found: C, 64.75; H, 4.85; N, 10.45.

6-(4-Chlorophenyl)-3-(2,3-dihydroxypropyl)-7-phenyl-3H-pyrrolo[2,3-*d*]pyrimidin-4(7H)-one 3e

Yield (71%); m.p.310-312^{0}C; IR (KBr) ν_{max} [cm^{-1}]: 1683(CO), 3428(OH); ^{1}H NMR (DMSO) δ ppm: 2.50 (s, 1H, OH), 2.71-2.73(d, 2H, N-CH_2), 2.87-2.89(d, 2H, OH-CH_2), 3.33-3.42(m, 1H, CH), -6.84(s, 1H, $H_{pyrrole}$), 7.18-7.86(m, 9H, $H_{arom.}$), 8.04 (s, 1H, $H_{pyrimidine}$); ^{13}C NMR (DMSO) δ ppm : 158.06, 149.54, 144.44, 135.78, 134,29. 132.24, 130.06, 129.83, 128.98, 128.32, 128.26, 128.07, 120,58, 108.48, 103.12, 63.71, 61.90, 45.56; MS m/z [%]: 395.05 [M$^+$] (16.96).397.20[M^{+2}] (11.28). Anal.Calcd. For $C_{21}H_{18}ClN_3O_3$ (395.84): C, 63.72; H, 5.58; Cl, 8.96; N, 10.62.Found: C, 63.95; H, 5.35; Cl, 8.84; N, 10.35.

6-(4-Chlorophenyl)-3-nonyl-7-phenyl-3H-pyrrolo[2,3-*d*]pyrimidin-4(7H)-one 3f

Yield (68%); m.p.280-282^{0}C; IR (KBr) ν_{max} [cm^{-1}]:1681 (CO); ^{1}H NMR (DMSO) δ ppm: 0.74-0.83 (t, 3H, CH_3), 1.28-1.66 (m, 10H, $5CH_2$), 2.49-2.58 (m, 2H, N-CH_2CH_2), 3.13-3.44 (t, 2H, N-CH_2), 6.88 (s, 1H, $H_{pyrrole}$), 7.10-7.4 8 (m, 9H, $H_{arom.}$), 8.19 (s, 1H, $H_{pyrimidine}$); MS m/z [%]: 447 [M$^+$] (25.90). Anal.Calcd. for $C_{27}H_{30}ClN_3$ O (448): C, 72.39; H, 6.75; Cl, 7.91; N, 11.19.Found: C, 72.45; H, 6.25; Cl, 8.11; N, 11.25.

3-{2-(2-Hydroxyethoxy)ethyl}-6-(4-bromophenyl)-7-phenyl-3H-pyrrolo[2,3-*d*]pyrimidin-4(7H)one 3g

Yield (72%); m.p.316-317^{0}C; IR (KBr) ν_{max} [cm^{-1}]:1689 (CO), 3420(OH); ^{1}H NMR (DMSO) δ ppm: ^{1}H NMR ($CDCl_3$) δ ppm: 2.31 (s, 1H, OH), 3.07-3.15 (t, 2H, N-CH_2), 3.45-3.56 (d, 2H, O-CH_2CH_2OH), 3.61-3.70 (d, 2H, N-CH_2CH_2), 3.75-3.80 (t, 2H, CH_2OH), 6.83 (s, 1H, $H_{pyrrole}$), 7.23-7.86 (m, 9H, $H_{arom.}$), 8.13 (s, 1H, $H_{pyrimidine}$); MS m/z [%]: 454.30 [M$^+$] (73.96).Anal.Calcd. For $C_{22}H_{20}BrN_3O_3$ (454.32): C, 58.16; H, 4.44; Br, 17.59; N, 9.25.Found: C, 58.55; H, 4.35; Br, 17.84; N, 9.37.

6-(4-Bromophenyl)-3-(2,3-dihydroxypropyl)-7-phenyl-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one 3h

Yield (78%); m.p.304-306^{0}C; IR (KBr) ν_{max} [cm^{-1}]:1683 (CO), 3408(OH); ^{1}H NMR (DMSO) δ ppm: 2.48 (s, 1H, OH), 2.69-2.73 (d, 2H, N-CH_2), 2.84-2.89 (d, 2H, OH-CH_2), 3.30-3.46 (m, 1H, CH), 6.82 (s, 1H, $H_{pyrrole}$), 7.17-7.87 (m, 9H, $H_{arom.}$), 8.12 (s, 1H, $H_{pyrimidine}$); MS m/z [%]: 439 [M$^+$] (36.80).Anal.Calcd. For $C_{21}H_{18}BrN_3O_3$ (440.29): C, 57.29; H, 4.12; Br, 18.15; N, 9.54.Found: C, 57.55; H, 4.35; Br, 18.44; N, 9.37.

6-(4-Bromophenyl)-3-nonyl-7-phenyl-3H-pyrrolo [2,3-*d*]pyrimidin-4(7H)-one 3i

Yield (78%); m.p.304-306^{0}C; IR (KBr) ν_{max} [cm^{-1}]:1686 (CO); ^{1}H NMR (DMSO) δ ppm: 0.70-0.82 (t, 3H, CH_3), 1.22–1.64 (m, 10H, $5CH_2$), 2.48-2.55 (m, 2H, CH_2), 3.11-3.41 (t, 2H, N-CH_2), 6.89 (s, 1H,$H_{pyrrole}$), 7.10-7.45 (m, 9H, $H_{arom.}$), 8.18 (s, 1H, $H_{pyrimidine}$); ^{13}C NMR (DMSO) δ ppm : 207.69, 206.64, 186.81, 168.81, 131.24, 130.47, 130.08, 129.20, 128.20, 125.65, 120.55, 108.56, 103.43, 61.01, 58.75, 45.45, 43.02, 32.47, 31.21, 28.55, 25.88, 22.01, 13.86; MS m/z [%]: 491.10 [M$^+$] (38.72), 493 [M^{+2}] (9.35).Anal.Calcd. For $C_{27}H_{30}BrN_3O$ (492.45): C, 65.85; H, 6.14; Br, 16.23; N, 8.53.Found: C, 65.55; H, 6.35; Br, 16.43; N, 8.31.

3. Results and Discussion

Chemistry

6-Substituted-7-phenyl-3,7-dihydropyrrolo [2,3-*d*] pyrimidin-4-one derivatives **2a-c,** the key intermediate for synthesis of title compounds **3a-i** (Scheme 1) was prepared by addition of formic acid to **1a-c** and refluxed the mixture for 4 hr. The IR spectrum of **2a** showed absorption bands at 3427 & 1668 cm^{-1} assigned to imino (NH) and carbonyl (CO) groups respectively. Furthermore, its ^{1}H NMR spectrum showed a singlet at δ 8.10 ppm & 10.10 ppm indicated the formation of pyrimidine ring and imino (NH) respectively confirmed the structure. Mass spectrum of **2a** showed molecular ion peak at m/z 287 corresponding to its molecular formula $C_{18}H_{13}N_3O$.

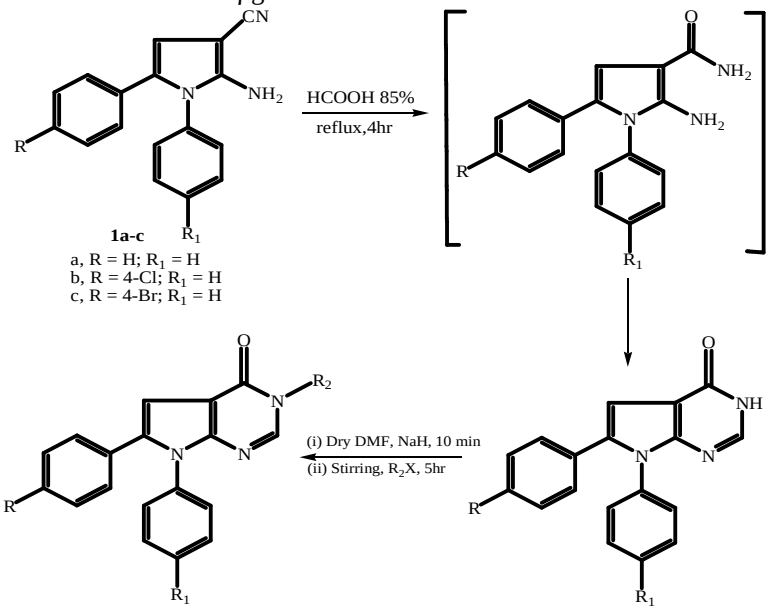
Compounds **2a-c** in dry DMF added sodium hydroxide and the mixture stirred at room temperature for 20 min and then the alkyl halide was added and refluxed to afford **3a-i**. Completion of the reaction was monitored by TLC. The title compounds **3a-i** was obtained in good yields (64 – 78%). The IR

spectrum of **3c** revealed the absence of the imino group (NH) and the appearance of absorption band at 1684 cm^{-1} for (CO) only. It's ^1H NMR spectrum also showed two triplet bands at δ 0.71–0.89 ppm & δ 3.19–3.43 ppm for methyl group (CH_3) and (N-CH_2) respectively. Also, the presence of characteristic multiplet at δ 1.20–1.67 ppm (5 CH_2) and δ 2.45–2.59 ppm (CH_2). All structures were assigned by their mass spectrum and elemental analysis.

Biology

Antiviral activity: There are many compound that synthesized, were shown to inhibit replication of both NDV virus and H5N1 virus in SPF (ECE's) when compared with "Ribavirin" (reference antiviral drug). The compounds **1a-c, 2a, 2c** showed good most anti Newcastle (ND) activating epically **1a,2a** are the most potent with MIC 2.6 &2.7µgm /ml respectively as shown in Table (1). Also, compounds **3a, 3c, 3g, 3h, 3i** showed a good anti (H5N1) where **3a, 3b, 3g, 3h,** are highly potent with MIC 3.8 , 3.6 , 4.5 and 4.1 µgm /ml respectively as shown in Table (2) .

Antibacterial activity: The in vitro antibacterial activating of different synthesized compound against two gram-negative bacteria [*Echerichia coli* ATCC 25992, *Salmomella typhimurium* ATCC 14028] were exhibited various levels of antibacterial effect against tested bacterial strains, as compared with reference antibiotic "Suttrium" where they produce a large and clear zone of inhibition when sensitive, organisms come in contact with the susceptible antimicrobial compound **1a-c, 2a-c, 3a-b, 3g-h** showed antibacterial effect where **1a, 1b, 2a, 2b** are the most potent compounds with MIC 2.6, 2.9, 2.8, 3.2 µgm/ml respectively as shown in table (4).



1a-c
a, R = H; R_1 = H
b, R = 4-Cl; R_1 = H
c, R = 4-Br; R_1 = H

3a-i
a, R = H; R_1 = H; R_2 = -$CH_2CH_2OCH_2CH_2OH$
b, R = H; R_1 = H; R_2 = -$CH_2CHOHCH_2OH$
c, R = H; R_1 = H; R_2 = -$(CH_2)_8CH_3$
d, R = 4-Cl; R_1 = H; R_2 = -$CH_2CH_2OCH_2CH_2OH$
e, R = 4-Cl; R_1 = H; R_2 = -$CH_2CHOHCH_2OH$
f, R = 4-Cl; R_1 = H; R_2 = -$(CH_2)_8CH_3$
g, R = 4-Br; R_1 = H; R_2 = -$CH_2CH_2OCH_2CH_2OH$
h, R = 4-Br; R_1 = H; R_2 = -$CH_2CHOHCH_2OH$
i, R = 4-Br; R_1 = H; R_2 = -$(CH_2)_8CH_3$

2a-c
a, R = H; R_1 = H
b, R = 4-Cl; R_1 = H
c, R = 4-Br; R_1 = H

Scheme 1. Synthesis of pyrrolo[2,3-*d*]pyrimidine derivatives **3a-i**

Conclusion

In summery, we have described an efficient synthesis for preparation of new pyrrolo[2,3-*d*]pyrimidine derivatives .These products were evaluated in vitro for their antiviral and antibacterial activities. It is good to say that the compounds **1a-c, 2a-c,** are shown highly antiviral activity towards Newcastle Disease Virus (NDV) where **1a, 2a** are highly potent.

Also compounds **3a-d, 3g-i** are shown highly antiviral activity towards Avian Influenza virus (H5N1) where **3a, 3b, 3g, 3h** are highly potent.

And it is good to be improved antibacterial activity was observed for most of the compounds against gram-negative bacteria used in the study.

Especially the compounds **1a-c, 2a-b, 3a, 3g, 3h** are shown highly antibacterial activity.

Finally, the outcome of these study is the possibility of using of such pyrrolo[2, 3-*d*]pyrimidines derivatives in the production of effective antiviral drug and in vaccine production after applying the known roles.

It is the first record to use these new compounds against highly pathogenic avian influenza virus (H5N1) causing "Bird Flu", and against Newcastle Disease Virus (NDV).

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Perceived Self-efficacy and Commitment to an Exercise in Patients with Osteoporosis and Osteoarthritis

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Abstract: Exercise remains a central health behavior in improving osteoporosis and osteoarthritis. A convenience sample of patients with a clinical diagnosis of osteoporosis (75) and osteoarthritis (75) was recruited. Their mean age±SD was 52.9±7.2 and 50.0±13.1, respectively. Obtained results revealed that osteoporotic patients had significantly higher mean scores of commitment to exercie, and higher exercise self-efficacy, while the mean score of exercise benefit was higher in the osteoarthritis group. In addition; a significant positive correlations were found between commitment and perceived self-efficacy scores, and between self-efficacy and perceived lack of barriers and exercise benefits in both groups. In regression analysis, age was a negative predictor for commitment in osteoporosis group, while self-efficacy and lack of barriers were positive predictors. In osteoarthritis group, self-efficacy was the only positive predictor of commitment. It is recommended that the heath care professionals should adopt strategies for enhancing patient's self-efficacy, give special attention to perceived barriers to exercise, and tailor exercise interventions to the different needs and perceptions of the patients with osteoporosis and osteoarthritis. The aim of this study was to compare perceived self-efficacy, exercise benefits, exercise barriers, and commitment to exercise between patients diagnosed with osteoporosis and osteoarthritis, and assessing the influence of perceived exercise self-efficacy, exercise benefits, and barriers on commitment.
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Keywords: Osteoporosis; Osteoarthritis, Exercise, Self-efficacy, Commitment

1. Introduction:

Osteoporosis is a disease characterized by micro-architectural deterioration of bone tissue, low bone mass, enhanced bone fragility, and increased risk of fracture **(NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy, 2001)**. With increasing life expectancy, it is becoming a major health problem worldwide, with higher magnitude in developing countries **(Maalouf *et al,* 2007).** The major problem of osteoporosis is that many patients are not aware that they have the disease until a fracture occurs. Osteoporotic fractures affect the quality of life and are associated with premature mortality. Although the disease is serious, yet it is preventable by modifying certain aspects of lifestyle such as physical activity, diet, and the use of hormone replacement **(Sedlak *et al*., 2000)**.

Osteoarthritis or degenerative joint disease is the most common form of arthritis. It was estimated to be the 10th leading cause of non-fatal burden in the world in 1990 **(World Health Organization, 2002)**. It is also responsible for 81% of hip replacements and 93% of knee replacements. It usually attacks the weight-bearing joints striking most often the hips and knees. Osteoarthritis occurs when cartilage deteriorates leaving the bones to grind against each other. This causes the bones to degenerate, resulting in pain and stiffness. As the condition worsens, loss of mobility and, in extreme cases, dysfunction and deformity can occur **(American Academy of Physical Medicine and Rehabilitation, 2010)**. As osteoporosis and osteoarthritis are quite different, health professionals need to understand differences and similarities between the two groups in exercise-related perceptions as a basis for tailoring exercise interventions to the needs and perceptions of patients with these musculoskeletal conditions **(Shin *et al,* 2006)**.

An accepted strategy for decreasing the risk of musculoskeletal and connective tissue diseases is regular exercise. In people with osteoporosis, exercise affects bone density, size and shape, and thus improves mechanical strength **(Turner and Robling, 2003)**. It also stimulates osteoblastic activity, and suppresses bone resorption **(North American Menopause Society, 2002)**. Exercise has also been shown to improve coordination and improve postural stability, which decreases the risk of falls, in addition to improving general physical health and wellbeing **(Close and Glucksman, 2000;Mar, 2004; Spark, 2010)**. Exercise, as well may be one of the most effective therapies for reducing joint pain and improving functions in patients with knee and hip osteoarthritis **(Shin *et al,* 2006)**. Nonetheless, the

problem is that any positive gains in bone strength will be lost when the patient stops exercising, so that it is important that the exercise be regular and ongoing **(Spark, 2010).**

Participation in behaviors that affect health outcomes has been explained by a number of theoretical models. One such model is the Health Promotion Model (HPM), which describes the causal mechanisms for health promoting behavior that focus on increasing physical activity, exercise self-efficacy, exercise benefits and barriers, and commitment to a plan for exercise **(Pender *et al,* 2002)**. Self-efficacy acknowledges the human capacity for self-regulation and development of competencies in specific behavioral domains. Perceived self-efficacy is not a measure of the skills one has but a belief about what one can do under different sets of conditions with whatever skills one possesses. The Health Promotion Model proposes that commitment to a plan of action such as regular exercise is determined by the individuals' beliefs concerning their self-efficacy, outcomes or benefits as well as perceived barriers to action **(Shin *et al,* 2006)**.

Significance of the study:

The prevalence of both osteoporosis and osteoarthritis escalates as people age. The extrapolated statistics estimated the burden of osteoporosis in Egypt in 2010 as about 8 million cases out of 80 million populations, while estimated osteoarthritis to be around 6 million cases. Although many factors are related to the occurrence and progression of osteoporosis and osteoarthritis, exercise remains a central health behavior in their prevention. Perceived self-efficacy is another cognitive mediator that molds the shape of illness and may improve compliance with prescribed therapies and exercise regimens. This study is aimed at; comparing perceived self-efficacy, exercise benefits, exercise barriers, and commitment to exercise between patients diagnosed with osteoporosis and osteoarthritis; and assessing the influence of perceived exercise self-efficacy, exercise benefits, and barriers on commitment to exercise in each group.

Research hypotheses

There is a difference between perceived self-efficacy, exercise benefits, exercise barriers, and commitment to a plan for exercise in patients diagnosed with osteoporosis and osteoarthritis.

Perceived self-efficacy is positively related to commitment to an exercise among patients diagnosed with osteoporosis and osteoarthritis.

Perceived benefits are positively related to commitment to an exercise among patients diagnosed with osteoporosis and osteoarthritis.

Perceived lack of barriers is positively related to commitment to an exercise among patients diagnosed with osteoporosis and osteoarthritis.

2. Subjects and Methods

Research design:

A comparative cross-sectional analytic research design was utilized in this study.

Research setting:

The study was conducted at the Physiotherapy Department and Outpatient Orthopedic Clinic of the Maternity Hospital, and Ain Shams University Hospitals.

Study subjects:

The participants in this study consisted of a convenience sample of 75 patients with a clinical diagnosis of osteoporosis and 75 patients with osteoarthritis, recruited from the study settings. The inclusion criteria were clinical diagnosis of osteoporosis or osteoarthritis and having the physical capacity to exercise. Exclusion criteria were, patients having both diagnoses, or with conditions making them unable to exercise such as cardiovascular disease as hypertension, decompansated heart failure, ischemic heart disease, or metabolic conditions as diabetes mellitus or thyroid problem, advanced renal – liver disease, severe anemia, obesity, CNS stroke, as well as exacerbated arthritis or recent fracture.

Tools of the study:

Four tools were utilized to collect data related to this study. They were all in Arabic language and self-administered.

Perceived Exercise Self-efficacy Scale: developed by Bandura (1997) and psychometrically evaluated by Shin *et al* (2001) to measure perceived self-efficacy. The scale has a total of 18 items ranging in 10-unit intervals from (0%) cannot do, through intermediate degrees of assurance (50%) moderately certain can do, to complete assurance (100%) certain can do. The respondent should rate him/herself, from 0% to 100%, how confident he/she is able to perform exercise routines regularly (three or more times a week) under various circumstances such as ‘during bad weather’. It was scored as an average of the patient’s response to the 18 items.

Exercise Benefits and Barriers Scale: used to determine the respondent's perceptions concerning the benefits of and barriers to participating in exercise. It was adopted from Sechrist *et al* (1987) and psychometrically evaluated by Jang and Shin (1999) for the validity of its constructs and for internal consistency and test-retest reliability. The scale has 43 items, 29 benefits and 14 barriers. They are rated on a four-points Likert scale ranging from strongly disagree (1) to strongly agree (4). Barrier scale items are reverse scored. The scale has separate

benefit and barrier scores. The scores are converted into percent scores to facilitate comparisons.

Commitment to an Exercise Scale: developed by Pender (1996) and psychometrically evaluated by Shin *et al* (2003), and adapted for cultural appropriateness by the researchers. The scale has 20 items with a three-point rating scale ranging from never (1) to often (3). The scale is scored as a total average for each patient, and also converted into percent scores to facilitate comparisons.

Exercise documentation record: developed by the researchers to document patient's adherence to the exercise. Its first section involves patient's demographic characteristics as age, gender, marital status, working status, educational level, in addition to weight, medical history, and present complaint. The second section was for documentation of information regarding the type of exercise practiced e.g (walking, cycling, swimming, others), addition of another type of exercise, the frequency of exercising as days per week and duration of exercise as minutes per day, the intensity of the exercise (how hard the patient perceives the exercise according to Borg Scale). It scored from (1) not hard at all to (10) extremely hard, in addition to the cumulative exercise time in minutes, and the rate of progression, and adding another type of exercise.

Procedures

The investigators went through literature review to adopt and finalize the study tools. Exercise was defined in this study as performing regular physical activity to improve fitness and health. Experts' advice was sought to ensure content relevance, clarity, and correctness. Administrative consent for study conduction was obtained from the directors of Ain Shams University Hospitals. Participants were given a full explanation of the study aims and procedures. Verbal consent was obtained by each patient prior to completing the study instruments. During data collection, one researcher was available at least to assist the study participants in explanations of the meaning of questions. Questionnaires took about 30–40 min for the subject to complete. Data was collected from June 2006 to June 2007.

Statistical analysis

Data entry and statistical analysis were done using SPSS 16.0 statistical software package. Quantitative continuous data were compared using Student t-test. When normal distribution of the data could not be assumed, the non-parametric Mann-Whitney test was used instead. Pearson correlation analysis was used for assessment of the inter-relationships among various scores. Multiple stepwise regression analysis was used to identify the independent predictors of the commitment score.

3. Results

As shown in table (1); a significant difference was found between the two groups regarding gender ($p=0.003$) and marital status ($p=0.002$). In addition; it is evident that the osteoporosis group had a higher percentage of females, and less unmarried subjects. On the other hand the two groups had similar age distribution, working status, and educational level.

As shown in table (2); the practice of walking was statistically significantly higher in the osteoarthritis group, and a higher percentage of them were adding two or more other types of exercise (26.7%), whereas patients in the osteoporosis group had higher physiotherapy exercises practice ($p<0.001$). it also, indicates that the osteoarthritis group perceived exercise as more intense compared to the osteoporosis group, and they showed lower cumulative duration of exercise; these differences were statistically significant ($p<0.001$).

As shown in table (3); osteoporotic patients had significantly ($p<0.001$) higher mean scores of commitment to plan, and exercise self-efficacy. Conversely, the mean score of exercise benefit was higher in the osteoarthritis group ($p<0.001$). Moreover, the addition to exercise level was significantly ($p<0.001$) higher in the osteoporosis group as 41.3% of them reported adding a high level of exercise (muscle stretching plus strengthening exercise twice a week).

As shown in table (4); a significant positive correlations between commitment and perceived self-efficacy scores in both groups. Additionally, the self-efficacy score was statistically significant positively correlated to perceived lack of barriers, and perceived exercise benefits in both groups. These correlations were stronger in the osteoporosis group. Also, statistically significant positive correlations were revealed between the scores of commitment and lack of barriers only in the osteoporosis group.

As shown in table (5); the best fitting multiple linear regression models for commitment scores in the osteoporosis group, age was a statistically significant negative predictor, whereas the scores of self-efficacy and lack of barriers were statistically significant positive independent predictors. As indicated by the value of r-square, the model explains 46% of the variation in the commitment score. In the osteoarthritis group, the score of self-efficacy was the only statistically significant positive independent predictor of commitment, but it explained only 16% of the variation in commitment score as the value of r-square shows.

Table (1): Socio-demographic characteristics of patients in the osteoporosis and osteoarthritis groups

	Group				X^2	p-value
	Osteoporosis (n=75)		Osteoarthritis (n=75)			
	No.	%	No.	%		
Age (years):						
<50	23	30.7	35	46.7		
50+	52	69.3	40	53.3		
Range	40.0-82.0		17.0-76.0			
Mean±SD	52.9±7.2		50.0±13.1		U=0.98	0.32
Gender:						
Male	17	22.7	34	45.3		
Female	58	77.3	41	54.7	8.59	0.003*
Marital status:						
Married	73	97.3	61	81.3		
Unmarried	2	2.7	14	18.7	10.07	0.002*
Working status:						
Employee	16	21.3	26	34.7		
Worker	13	17.3	16	21.3	4.83	0.09
Unemployed/housewife	46	61.3	33	44.0		
Educational level:						
Illiterate	12	16.0	12	16.0		
Read/write	10	13.3	14	18.7		
Basic	19	25.3	11	14.7	3.02	0.55
Secondary	27	36.0	30	40.0		
University	7	9.3	8	10.7		

(*) Statistically significant at p<0.05 *(U) Mann Whitney test*

Table (2): Types and levels of physical exercise practiced by patients in the osteoporosis and osteoarthritis groups

	Group				X^2	p-value
	Osteoporosis (n=75)		Osteoarthritis (n=75)			
	No.	%	No.	%		
Type of exercise:						
Walking	11	14.7	70	93.3	93.42	<0.001*
Cycling	0	0.0	2	2.7	Fisher	0.50
Gym	0	0.0	15	20.0	16.67	<0.001*
Physiotherapy	65	86.7	23	30.7	48.50	<0.001*
Adding another type of exercise to the exercise plan:						
1	74	98.7	55	73.3		
2+	1	1.3	20	26.7	19.99	<0.001*
Frequency of exercise (no. of days/week):						
Range	2.0-6.0		2.0-3.0			
Mean±SD	2.9±0.6		3.0±0.2		U=3.30	0.07
Duration of exercise (no. of minutes/day):						
Range	10.0-40.0		5.0-30.0			
Mean±SD	24.0±6.2		20.4±6.1		t=3.59	<0.001*
Exercise intensity (Borg scale):						
Range	2.0-7.0		3.0-9.0			
Mean±SD	4.4±1.3		5.6±1.5		t=5.26	<0.001*
Cumulative duration index:						
Range	90.0-810.0		60.0-630.0			
Mean±SD	393.6±162.2		270.6±118.4		H=21.23	<0.001*

(*) Statistically significant at p<0.05 (U) Mann Whitney test (t) Student t-test

Table (3): Scores of commitment, perceived self-efficacy, lack of barriers and benefits, and progress of exercise among patients in the osteoporosis and osteoarthritis groups

	Group				X^2	p-value
	Osteoporosis (n=75)		Osteoarthritis (n=75)			
	No.	%	No.	%		
Commitment to exercise score:						
Range	25.0-100.0		5.0-95.0			
Mean±SD	77.7±16.0		62.3±15.9		5.91	<0.001*
Perceived exercise self-efficacy score:						
Range	33.9-82.2		11.1-75.0			
Mean±SD	60.5±12.9		43.5±13.0		8.03	<0.001*
Perceived lack of barriers score:						
Range	41.1-78.6		42.9±85.7			
Mean±SD	63.7±6.9		61.5±9.5		U=3.56	0.06
Perceived exercise benefits score:						
Range	60.3-93.1		52.6-94.8			
Mean±SD	73.3±5.5		78.0±8.9		18.67	<0.001*
Progress:						
Limited	27	36.0	24	32.0		
Moderate	43	57.3	44	58.7	0.52	0.77
High	5	6.7	7	9.3		
Addition of extra exercise level						
Muscle stretching	20	26.7	50	66.7		
Muscle stretching plus strengthening exercise once a week	27	32.0	19	25.3	30.33	<0.001*
Muscle stretching plus strengthening exercise twice a week	31	41.3	6	8.0		

(*) Statistically significant at p<0.05 (U) Mann Whitney test

Table (4): Correlation matrices of various scores of patients in the osteoporosis and osteoarthritis groups

	Pearson correlation coefficient					
	Osteoporosis group n=75			Osteoarthritis group n=75		
	Commitment to plan score	Exercise self-efficacy score	Lack of barriers score	Commitment to exercise score	Exercise self-efficacy score	Lack of barriers score
Exercise self-efficacy score	0.593**	-	-	0.404**	-	-
Lack of barriers score	0.549**	0.599**	-	0.085	0.278*	-
Exercise benefits score	0.490**	0.603**	0.187	0.156	0.420**	0.069

(**) Statistically significant at p<0.01

Table (5): Best fitting multiple linear regression models for scores of commitment to exercise in the osteoporosis and osteoarthritis groups

	Beta coefficients			t-test	p-value
	Unstandar-dized	Standard error	Standardized		
Osteoporosis group					
Constant	39.49	19.33	39.49	2.042	0.045*
Age (years)	-0.52	0.20	-0.52	-2.589	0.012*
Self-efficacy score	0.47	0.14	0.47	3.441	0.001*
Lack of barriers (score)	0.59	0.26	0.59	2.331	0.023*
	r-square: 0.46 Model ANOVA: F=20.28, p<0.001 Variables excluded by model: sex, education, marital status				
Osteoarthritis group					
Constant	40.74	5.97		6.828	<0.001*
Self-efficacy score	0.50	0.13	0.40	3.774	<0.001*
	r-square: 0.16 Model ANOVA: F=14.24, p<0.001 Variables excluded by model: age, sex, education, marital status				

4. Discussion

Despite recent pharmacologic advances in the prevention and treatment of osteoporosis and osteoarthritis, the diseases remain incurable. Effective disease management ultimately lies in the hands of the individual patient, who must take responsibility for key health behaviors related to bone and joint health **(Gold and Silverman, 2004).** Self-efficacy is a treatment-responsive mediator of illness and has been shown to improve the clinical outcome for patients with several types of chronic disease, including arthritis and osteoporosis **(Kligler and Lee, 2004).**

In order to make the comparisons between the two groups valid, their comparability regarding the factors that could affect commitment and self-efficacy was examined. They had similar age, educational level, and working status. However, the osteoporotic group had more women, and higher percentage of married subjects. This is expected given the known higher prevalence of osteoporosis among women in this peri-menopausal age group as indicated by **Sedlak *et al*. (2000)**. Similarly, **Al-Hussain (2007)** mentioned that osteoporosis is a growing health problem in Middle East, with one out of each three woman above fifty years of age suffering the disease. Additionally, the rate among women has been estimated to be twice that of men **(Bone and Joint Health Institute, 2010)**.

Concerning exercise practice, the current study findings demonstrated significant differences between osteoporosis and osteoarthritis groups. In the osteoporosis group, physiotherapy was the predominant type of exercise, while almost all osteoarthritis group patients were practicing walking. It was also noticed that more osteoarthritis patients were practicing more than one type of exercise. However, duration of exercises as minutes per day, cumulative duration index were significantly lower in the osteoarthritis group, with a more intense exercise perception. These differences between the two groups might be attributed to the presence of pain and fatigue as main barriers to exercise in osteoarthritis patients. In congruence with this, a number of studies confirmed that the disease-associated symptoms as pain and fatigue can pose obstacles to enhance physical activity in osteoarthritis **(Leveille *et al*, 2002, Avlund *et al*, 2003, Murphy *et al*, 2008)**.

The present study findings point to a higher commitment to exercise in both groups with significant differences between them, where osteoporotic patients had significantly higher scores of commitment and self-efficacy, osteoarthritis patients had higher perception of exercise benefits. These findings were in agreement with **Shin *et al***

(2006) who showed more commitment to exercise among women with osteoporosis, compared to those with osteoarthritis. This finding was explained by the absence of pain among osteoporotic patients, which is a hindering factor to commitment among osteoarthritis patients. This also might explain the higher percentage of osteoporotic patient who could increase their exercise level.

Therefore, the commitment to exercise noticed among patients in the present study, and their maintenance of regular exercise is a health seeking behavior fostered by their perception of self-efficacy (which was significantly higher in osteoporotic patient). This might explain the higher commitment among subjects in both groups, as self-efficacy turned to be an independent predictor for commitment regardless the type of disease. A number of previous studies have similarly revealed that self-efficacy was the best predictor variable for intention to exercise, a construct somewhat similar to commitment to regular exercise activity (**Wu and Pender, 2002). Shine *et al*, (2006),** also demonstrated that self-efficacy was the most influential variable on commitment to a plan for exercise. **Kim (2001)** interpreted this positive correlation between self-efficacy and commitment to exercise plan by the fact that highly self-efficacious individuals exert greater efforts to master health-promoting behavior. Moreover, a strong sense of efficacy enhances human accomplishment and personal wellbeing, and encourages setting challenging goals and maintain strong commitment to them **(Laffrey, 2000)**.

On the other hand, the self-efficacy may be secondary to their perceived benefits of exercise as showed in the present study that both groups achieved relatively high benefits score, although the osteoarthritis group had significantly higher mean compared to osteoporosis group. This might be referred to different beneficial effect of exercise in both groups especially those with osteoarthritis where the benefit is more noticeable as it may appear in the form of pain relief and more fitness to exercise, compared to the osteoporosis group patients who usually have no such complaints, which foster osteoarthritis patients to commit to regular exercise activity. These beneficial changes might also be responsible about increasing the self-efficacy among osteoarthritis patients. Thus, their perception of exercise benefit was positively correlated to their self-efficacy scores and not to commitment score as in osteoporosis patients. In agreement with these findings, a systematic review of randomized clinical trials of the effectiveness of exercise therapy in patients with knee osteoarthritis, the researchers found a small to moderate beneficial effect on pain, with a moderate to large beneficial effect on patient's global assessment of wellbeing **(VanBaar etal., 1999)**.

The present study showed no statistical significant difference between the two groups regarding perceived lack of barriers, while a strong positive correlation was revealed between perceived lack of barriers and self-efficacy in both groups. This was explained by **Kim (2001)** who stated that the highly self-efficious individual could persist longer in the face of obstacles to health promoting behavior. Moreover, the perceived lack of barriers to exercise as shown in the present study is an important factor contributing to compliance and commitment to exercise in osteoporosis group. This was evident in a review of the results of 38 explanatory and predictive studies that tested Health Promotion Model, the variables that were significant in predicting health promoting behaviors in over 60% of these studies included the perceived lack of barriers **(Pender *et al*, 2002)**, which was similar to the present study findings that lack of barriers was one of the independent predictors to commitment in osteoporosis group.

Additional predictors of commitment among osteoporosis patients were the younger age. The advancing age is certainly associated with more severity of the osteoporosis problems, in addition to more fatigue sensation, which may decrease patient's level of commitment to exercise. In agreement with this finding, a negative relationship between age and commitment to exercise plan was achieved by **Noel and Pugh (2002)** and **Kruger *et al* (2005).** Our findings were also in congruence with the Rosenstock's Health Belief Model (HBM), which proposes that perceived benefits and barriers to action and self-efficacy were among factors influencing illness-preventing behavior **(Sedlak *et al*., 2000)**.

Conclusion and Recommendations

The current study findings lead to the conclusion that perceived exercise self-efficacy is a major influential factor associated with commitment to exercise in both osteoporosis and osteoarthritis patients. Moreover, patient's scores of commitment to exercise, self-efficacy, perception of lack of barriers, and benefits from exercise are positively inter-correlated.

Therefore, it is recommended that the heath care professionals should adopt strategies for enhancing patients' self-efficacy through motivational counseling, which would lead to more effective health promotion programs for patients with osteoporosis and osteoarthritis. Perceived barriers to exercise should be given a special attention in counseling these patients. Tailoring exercise

interventions to meet different needs and perceptions of the patient with osteoporosis and osteoarthritis, which can be of help in reducing these barriers.

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The impact of premenstrual syndrome on social Participation of the 25-35 year old female staffs of Baft city (Kerman Province) in 2010

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Abstract : Development is not materialized without the women's contribution, so that preparing an appropriate ground for different activities of the women and fighting against all barriers of women participation in socio-economic and political activities is essential. One of the barriers is the existence of the Premenstrual Syndrome (PMS) in women. PMS is a set of disturbing signs which are experienced by many people some days before menstruation. Many of pro-women thinkers believe that PMS is a psychological disturbance being created by medical science to treat a natural biologic process, so that to prevent of the women interference in heavy and strong activities in the society. In order to determine the existence of PMS and its consequences, a study was done on 130 women in 25 to 35 years old who were the female employed staff in the public offices in Bam city. It was a survey and in order to collect data, in addition to questionnaires, books and other documents were also used. The results show that the percentage of affliction with PMS was 94%. The affliction with depression resulting from the syndrome was reported in 70% of the individuals and those afflicted with agony were 64%. This high pain of PMS affliction along with the consequences resulting from the physical signs, distress and depression can have a threatening factor for the women's health and a pretext to make employers disappointed of employing women. Journal of American Science 2011; 7(8):324-328]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: Women, Premenstrual Syndrome, Distress, Depression, Social Participation

Introduction:

The term Premenstrual Syndrome (PMS) is a periodical disturbance which entangles feelings, behaviours and physics of the body in the pre-stage of menstruation. The PMS is an important clinical situation which affects the majority of women. Even the percentages of women are afflicted with the PMS such that its damages can have impacts on the productivity of the afflicted individuals in working environment, home, social relations and their social life. It can endanger their mental and social health as well. This syndrome with a high load resulting from distress and depression and physical pains such as tiredness, muscular pains and etc is a barrier for the pervasive contribution of women in different dimensions including their social contribution in the society.

Though, long years have passed since the identification of this syndrome, but only in recent years, attention has been focused on the set of its full signs and that is due to the women involvement in jobs outside home and undertaking the critical positions. Notwithstanding, the people do not understand that in fact the women in the period of affliction with PMS are patients with a high need to care and should be helped to recover and they are considered as incapable, invalid and face gender discrimination and removed from the social science.

General purpose

Identifying signs of the PMS and its relation with the social relations of the 25-35 year old female staff of public offices in Baft city in 2008

Research questions:

1. Is there a relation between PMS and social relations of women?
2. Is there a relation between PMS and the emergence of depression?
3. Is there a relation between PMS and the emergence of distress?

Women and PMS

PMS can be defined as the cyclical return of a combination of physical and psychological changes or behaviours during the stages of leutel cycle of menstruation which involves the family, social or professional activities of the individual. Almost one percent of the women at the fertilization age are afflicted with PMS so that it endangers the patient' relations and inter-relations of the individual (Riyan, 2006, P. 65).

The signs of the PMS are diverse but in general they include the followings:

1. Body pain (headache, cramp, exhausting and etc.) 2. Water accumulation (increase of weight, inflation and painful breast), 3. Negative mood (depression, crying, feeling loneliness and irritation), 4. Autonomic reactions (cold perspiring, giddy,weakness and unconsciousness) 5. Beavertail changes (reduction of efficiency, difficulty in concentration and reduction of kinetic coordination,6.Somatisation status (feeling suffocated, pain in the breast, feeling the sound of bell in ears, blind spots in the visionary domain , blurred vision , shakiness and etc.) Spirov: 1992,. 65).

For the first time, this syndrome was explained by Frank. He find a creditable position through a report in which he used the term of premenstrual tension of an indefinable tension and increase of the epilepsy activities at the end of stage of leutel in a group of women being afflicted with epilepsy disorders. He reported that this tension is increased during he stages of leutel and with the start of menstrual bleeding it moves to remission (ibid, 611). About 3 to 5 percent of women with such observable signs or disenabling symptoms, according to psychological diagnosis, the Premenstrual Dysphoric Dirordere are true in their cases (Barrak, 2003, P. 223).

The high rate of committing suicide and violent actions such as violence committed by women occur in four days before menstruation and during the four days of menstrual period (Haid, 2004, P. 350). Forty five percent of the women workers in the industrial factories who became ill, forty six percent of the women who are accepted in mental centres are either in the period of 8 days before menstrual period starts or inside the menstrual period which indicate perhaps the increase of the distress in mothers in this part of the time (ibid). On the other hands, 8 days before menstrual period and the days of menstrual period form the 36 percent of the total days of a cycle. So, statistics which say for example 49 percent of violations by women takes place in this time period is by itself and considerably high (ibid, 350). It is estimated that 20 to 40 percent of women mention considerable difficulties in relation with their menstrual cycle. About 20 percent of them complaining some degrees of working disturbance and their routine jobs (Spirov, 1992. 115).

The social structure of PMS

The viewpoint of the advocates of women rights emphasizes on the gender roles based on external, social and environmental factors. The expression of feelings is exactly adjusted by social norms. Concerning most of the emotions such as love, sadness–expressing them by women is more acceptable as compared with men. The emotion of anger is an exception. The expression of anger by men has been borne but for women it is not so. A woman who expresses her anger violates the social norm (Haide, 2004, p. 357).

The expression of anger in women is not acceptable for these reasons because it interfere with their social role. The family role of women called them to prepare care and emotional support for others. The angry person can not behave affectionately and take care after and offer emotional support. This is also true in job roles and still anger interferes in job performance. Nobody wishes to be taken care after by an angry nurse. On the other hands, many of women are forced to become angry due to low level jobs, inequality in paying the wages vis-à-vis an equal work, violation and lack of access to high posts (ibid, 358).

So, in summary, many women feels anger or distress but to feel these emotions- emotions which are expressed less-is a serious deviation from social norms. This makes that women and her companions pursue an acceptable explanation for her expression of emotions. So, the issue of PMS is put forth. The PMS from the viewpoint of psychology or social structuralism as a reference is put forth. The woman experiences a specific emotion or expresses it. This woman refers this specific emotion to something. If the emotion is an unacceptable social emotion such as anger or distress, she and the others search for acceptable social references and the society thinks that PMS is a reference and easily accessible. She surprisingly does not become angry, but she is in the temporary stage of mental disorder, i.e. PMS. Her emotions with a little further reference do not anymore violate social norms. But at the same time, any real feelings from here real anger towards perhaps husband or boss is also removed (ibid, p. 358).

Research methodology:

The research methodology of this research is survey.

The tools and method of data collection:

In addition to questionnaire (questionnaire of physical symptoms, measuring the scale of clinician distress and depression and questionnaire of social relations, the library method has been also used.

Statistical community

All women of 25-35 years old being employed in Baft city (due to access to the statistical society, the sample volume was not used).

The method of data analysis

In this research, the descriptive and inferring statistics have been used. At the level of descriptive statistics, for the organizing and summarizing the data to be understandable and interlinking, descriptive tables were made (frequency distribution

and percentage). Furthermore, at the level of inferring statistics, the Khee Du calculation has been used.

The theoretical framework of the research

The theoretical framework of this research has used the theoretical views of Kert Lovin's psychology and sociology. Levin believes that his field theory aims at the link of the social living space and behaviour of the individual. He thinks that the behaviour of the individual is a function of this atmosphere which is understood through his needs and objectives (Turner, 1998, 78). Through this theory, some of the unnatural behaviours and states of human such as depression, alienation, anger, suicide are clarified. The concept of the conformity emphasises on coupling the individual variables with environmental variables. The more the individual expectations are in harmony with the expectations of the environment, the more will be the adaptability of individual and environment and the behaviour will be more nature and the environmental pressure on individuals will be also less (ibid).

Findings analysis

In order to study the impact of PMS on social contribution of the female staff of Baft city, 130 people of the employed female staffs of public offices were identified in Baft and responded to the related questionnaires.

The first objective in implementing these questionnaires was to study the spread of PMS among these women. A person was considered afflicted with PMS who has a score higher than 2 in the series of physical symptoms of PMS. 122 people (94 percent) of the 130 participants in this research were afflicted with PMS and 8 people (6) percent were not afflicted with the PMS.

The first assumption: Determining the relation between PMS and change in the social The conformity Table of change in the social changes versus the physical symptoms of PMS

Table1, calculations of independence test

Test statistic	14.29
P	0.003

With regard to the fact that–p obtained of the test of independence of two variables of those afflicted with the PMS syndrome and change in the social relations of women (0/003) is less than the meaningful level of (0/05), so the two variables are not separate from each other and change in the physical signs of PMS will cause reduction or increase of change in the social relations of the women.

The second assumption: Determining the relation between PMS and affliction with depression

Table2, calculations of independence test

Test statistic	13.85
P	0.003

With regard to the –p, the quantity resulting from the test of independence between two variables of affliction with PMS and intensity of depression (0/300) which is less than meaningfulness of (0/05), so it is concluded that the two variables of depression and PMS are not independent from each other and when the score of physical and PMS affliction, the depression goes up. Of the 8 people with non affliction with PMS, 87/5% had normal scores and were not afflicted with depression. But in the group afflicted with PMS, only 26% percent were not affiliated with depression and even 47 people (39%) were afflicted with an intensive and high depression.

The third assumption: Determining the relation between PMS and affliction with agony (distress)

The conformity Table of distress versus physical signs of PMS

Table 3, calculations of impendence test

Test statistic	12.8
P	0.047

With regard to the fact that–p of the obtained quantity from the independence test of affliction with PMS and increase of distress (0/047) which is less than significance of (0/05), so the two variables are not independent from each other and change in the physical signs of PMS causes change in reduction or increase of distress. 75% of the non-afflicted people with PMS had a natural score of distress but in the group afflicted with PMS, only 33/6% of the individuals were not afflicted with distress and had a natural score of distress. The score of intensive and very high in the group of afflicted with PMS allocated 33/6% of the scores related to the affliction with distress to themselves. But this score in the group which are not afflicted with PMS is zero. This means that in the group who are not afflicted with PMS, the affliction with an intensive or very high distress was not observed. But 41 people (34%) of the people afflicted with PMS were afflicted with a grave or very grave distress.

Conclusion and approaches

PMS is one of prevailing disorders of the fertilization years which along with menstrual pains

disturb the women life considerably. This syndrome exists in all cultures and has different impacts on social, economic, and professional life of women. This syndrome is observed among the women of 25 to 35 years old who are employed in the public offices in Baft city with a 94% prevailing rate. This syndrome with various difficulties which put ahead of this group leads to the spread of depression, stress and physical pains among them. It is such that with the affliction with PMS and moving up the scores of the physical signs of syndrome, the score of affliction with depression and distress goes up considerably. Of the 122 people afflicted with PMS, 68% (83 people) were afflicted with depression. 66% (81 people) were afflicted with distress and 82% (100 people) were afflicted with the change in social relations. So the PMS along with the grave pain resulting from the physical problems, depression, and distress is seen among the women of Baft. This has led to the reduction of efficiency, lack of concentration and tiredness resulting from muscular pains associated with depression and distress. This disease with a broad level and different consequences exist in that society but unfortunately, it is not considered as it is and has not received sufficient attention by authorities, whereas PMS is a difficult issue with a relatively simple solution. Because, the women with a slight attention can reduce this issue by correcting their food program, doing exercises, and … and they can even bring this difficulty into zero level. This will be possible only through planning on the side of officials to promote the knowledge of women and giving them further freedom of action to the employed women.

At the end, it is necessary to propose some suggestions to the authorities. Firstly, PMS is one of the complex issues of the social life, because half of the individuals of the society are suffering from it and faces its consequences. The officials should give sufficient attention to the social and psychological aspects of it in the social interaction among individuals.

Secondly, different social institutions including the Ministry of Health and Medical Education, Management and Planning Organization, etc. should support the researches in this area and help with further understanding of different issues of women afflicted with PMS who are a high percentage of the society i.e. half of the individuals of the society with high education, professional knowledge and capabilities. They play unique roles in the development of the society. The officials should forecast necessary approaches in policymaking and planning to reduce the unfavourable impacts of this disease.

Of the most important approaches, the followings may be mentioned:

- In those jobs which are done in shift forms, the women to be permitted to select their hard and full of stress shift based on their menstrual cycles. They must be trained that to transfer the stress producing part of their job to the week after menstrual period. Having relaxation in the week before menstrual period can be useful in the control of the outcomes of PMS.

- It is necessary to have the education of reconciliation with PMS as part of the educational activities of female personnel. They must become familiar with the scientific issues and ways to reduce the consequences of this disease. Because as long as the individuals are not familiar with a disease, they will not pursue treatment. The women should be taught that they must not change their meal hours, sleeping time and usual program of their activities some days before menstrual period. Maintaining usual order of these three issues help with internal and harmonic order of the body. They must be trained that it is necessary to sleep well (every night 8 hours) before or during menstrual period. Lack of sleeping, the reduction of serotonin in the body, exhaustion and irritation resulting from that will add fuels to PMS.

- Unfortunately, due to lack of public knowledge about this fact that it is necessary to be mindful of the women in PMS period in order to help them recover, the women are blamed for incapability and even face gender discrimination. If managers do not become aware of this preventive weakness, they will not be eager to employ women and the society will be deprived of the half of its skilful and expert forces. So arranging educational courses for managers and familiarizing them with this disease seems to be very necessary.

- The respectable officials should ban drinking teas and those holding caffeine and sugar in offices and should encourage women to consume fresh fruits.

Health is the pivot of development and women are axes of health. So paying attention to the health of the women is one of the most important axis of the development of the society, because a healthy human helps with the development of the society. We must treat the PMS with a high spread as one of the barriers in the path of women health and an obstacle for her participation in the society affairs. It has been rarely dealt with due to perhaps lack of awareness of it.

In fact, if we really wish to have the participation of the women in the process of sustainable development, and want to have it increase regularly, this objective will become only possible by facilitating the participation of women in social

activities. Otherwise, by putting forward mottos on the necessity of women participation and that the men and women's conditions are fully equal, no problem will b solved. Inequality between the conditions of competition of men and women in the social, economic, etc scene is not a phenomena of the recent time, and could be removed fast and easy. This inequality is the outcome of a long history which will not be solved only with superficial equality at the condition of unequal competition.

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Experimental Equality and Under Determination

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Abstract: During this century, there emerged from the philosophical analysis of scientific theories two results invested with broad epistemological significance By the 1920s, it was widely supposed that a perfectly general proof was available for the thesis that there are always empirically equivalent rivals to any successful theory. Secondly, by the 1940s and 1950s, it was thought that - in large part because of empirical equivalence - theory choice was radically underdetermined by any conceivable evidence. Whole theories of knowledge (e.g. , W. V. Quine's) (Fraassen. 1976) have been constructed on the presumption that these results were sound; at the same time, fashionable recent repudiations of the epistemic project (e.g., Richard Rorty's) have been based on the assumption that these results are not only legitimate, but laden with broad implications for the theory of knowledge. In this paper, we reject both the supposition of empirical equivalence and the inference from it to underdetermination. Not only is there no general guarantee of the possibility of empiricalk equivalent rivals to a given theory, but empirical equivalence itself is a problematic notion without safe application. Moreover, the empirical equivalence of a group of rival theories, should it obtain, would not by itself establish that they are underdetermined by the evidence. One of a number of empiricalk equivalent theories may be uniquely preferable on evidentially probative grounds. Having, argued for these conclusions in the first two sections, respectively, we shall propose, in section III of this paper, a diagnosis of the difficult that has impeded their recognition, and extract in attendant, positive moral for the prospects of epistemology.
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1. Introduction

This paper is concerned with the venerable dispute between realism and antirealism. In this paper we discuss that the aim of science is to discover truths about observables and unobservable which is "scientific realism." On this view, a proper reconstruction of scientific reasoning interprets accepting a scientific theory as believing it true. Science confirms the truth of claims concerning entities too small to be observed, such as subatomic particles or genes, or entities that are unobservable despite being large, such as the electromagnetic field, component forces, and my superego, just as it confirms the truth of predictions about observables that haven't yet been observed and perhaps never will be.

In this paper it is discussed that there are many varieties of antirealism. An anti-realist influenced by early logical positivism might believe that any scientific claim apparently purporting to describe unobservable is actually made true or false by observable facts alone and so we may be justified in believing it true. Alternatively, instrumentalism holds that a scientific claim or theory apparently purporting to describe unobservable has no truth-value. Rather, it is merely a piece of conceptual machinery - a device, made of cognitively meaningless marks, for generating empirical predictions from observations. In contrast to these non-literal construal of scientific theories, van Fraassen's version of scientific antirealism takes theoretical claims literally that is, as purporting to describe unobservable. An antirealist might contend that empirical evidence can never justify our believing in the truth of claims about unobservable - perhaps because many theories, disagreeing in what they say about unobservable, are empirically equivalent. Bellarmine intended to remind Foscarini of this argument for antirealism by me ntoining epicycles and eccentrics- different arrangements of the crystalline spheres posited as carrying the planets around the Earth. The ancients knew that these different arrangements generate exactly the same observables. This is the sort of argument for antirealism that Laudan and Leplin criticize. Some realists argue that to decide among empirically equivalent theories, scientists employ criteria of theory choice such as those discussed in the present paper. Van Fraassen defends antirealism without contending that it would be irrational to believe in the truth of claims about unobservable. He denies the realist view that it is rationally compulsory for scientists to believe in the approximate truth of the theories that they accept. Moreover, according to van Fraassen, a scientist's belief in a theory's truth - insofar as it goes beyondher belief in its empirical adequacy and her commitment to using that theory to

deal with new phenomena and to set her scientific agenda plays no role in her *scientific reasoning.* Belief in the theory may cause a scientist to come up with theoretical innovations that lead to greater empirical adequacy and may be important to a scientist's extra scientific pursuits. Van Fraassen's view that accepting a theory involves being committed to using the theory's resources in connection with future scientific tasks is taken even further by Sellars, who regards theoretical terms as observational terms in waiting. Furthermore, van Fraassen seems to believe that although rationality permits us to believe in the truth of various claims about unobservable, a genuinely empiricist attitude which isn't rationally compulsory would lead us to regard such belief as unworthy.

To forgo such belief and be agnostic about the reality of the unobservable posited by our best theories seems the fittest thing for the empiricist philosopher. Having looked at some varieties of antirealism, I would now like to examine briefly how realists and antirealists conceive of the structure of scientific theories. Hempel begins with the standard view of a scientific theory as consisting of a deductively closed, axiomatizable set of sentences forming three levels. At the bottom level arc reports of particular observable facts. The middle level consists of generalizations expressed entirely in the observational vocabulary Vu - the terms of which were well understood prior to any such theories being formulated. Interpretive sentences, a.k.a. "correspondence rules," "bridge principles", mediate inferences from sentences in Vb to sentences containing the theoretical vocabulary Vt, the terms of which apparently purport to refer to unobservable posited by the theory, and vice versa. The top level consists of theoretical laws mediating inferences between Vt sentences. On this layer-cake view, claims about unobservable predict and explain observations only indirectly: by entailing empirical generalizations that explain them directly. We establish the middle layer's uniformities by induction from our observations, logically prior to positing any unobservable. In short, only the cake's middle layer, not its top layer, touches the bottom. In contrast to Hempel's syntactic conception of scientific theories, this takes theories to be collocations of sentences, that is nonlinguistic entities representing all the possible worlds where the theory is true.

2. I Problems with Empirical Equivalence

A. Inducements to skepticism

The idea that theories can be empirically equivalent, that in fact there are indefinitely many equivalent alternatives to any theory, has wreaked havoc throughout twentieth-century philosophy It motivates many forms of relativism, both ontological and epistemological, by supplying apparently irremediable pluralisms of belief and practice. It animates epistemic skepticism by apparently underwriting the thesis of underdetermination. In general, the supposed ability to supply an empirically equivalent rival to any theory, however well supported or tested, has been assumed sufficient to undermine our confidence in that theory and to reduce our preference for it to a status epistemically weaker than warranted assent.

Specifically, this supposed ability is the cornerstone of arguments for the inscrutability of reference and the indeterminacy of translation, which together insulate the epistemic agent by challenging the objectivity of criticism on which an entire philosophical culture has depended. It has spawned prominent, contemporary versions of empiricism, including those of Quire, Bas van Fraassen, and J. D. Sneed, which belie the promise of science to deliver theoretical knowledge. It encourages conventionalism in geometry through Hans Reichenbach's invocation of universal forces. It questions the possibility of ordinary knowledge of other minds through the contrivance of the inverted spectrum. It blocks inductive generalization through the stratagem (it fashioning artificial universals to vie with natural kind, as, in Nelson Goodman's "grue" paradox, reducing the taws of apparent laws to mere entrenchment .

B. An argument against empirical equivalenece

We find the pervasiveness of this influence out of proportion to the conceptual credentials of the basic idea of empirical equivalence. by connecting three familiar and relatively uncontroversial theses, we can construct a simple argument to cast doubt on empirical equivalence in general, as a relation among scientific theories (and, by parity of reasoning, between any rival perspectives).

On the traditional view, theories are empirically equivalent just in case they have the same class of empirical, viz., observational, consequences (Laudan, 2000).A determination of empirical equivalence among theories therefore requires identifying their respective empirical consequence classes. As the empirical consequences of any statement are those of its logical consequences formulable in an observation language, these classes are (presumably proper) subsets of the logical consequence classes of theories. Central, therefore, to the standard notion of empirical equivalence are the notions of observational properties, the empirical consequences of a theory, and the logical consequences of a theory. We shall show that, when these concepts arc properly

understood, the doctrine of empirical equivalence loses all significance for epistemology.

Cur three familiar theses are these:

Familiar thesis 1, the variability of the range of the observable (VRO):

Any circumscription of the range of observable phenomena is relative to the state of scientific knowledge and the technological resources available for observation and detection.

In particular, entities or processes originally introduced by theory frequently achieve observable or "empirical" status as experimental methods and instruments of detection improve. Such variability applies to any viable distinction between observational and theoretical language (Leplin, 1996).

Familiar thesis 2, the need for auxiliaries in prediction (NAP):

Theoretical hypotheses typically require supplementation by auxiliary or collateral information for the derivation of observable consequences.

While direct derivability of statements bearing evidentially on theory is not in principle precluded, auxiliaries are generally required for the derivation of epistemically significant results (Laudon et al, 2006).

Familiar thesis 3, the instability of auxiliary assumptions (IAA):

Auxiliary information providing premises for the derivation of observational consequences from theory is unstable in two respects: it is defeasible and it is augmentable.

Auxiliary assumptions once sufficiently secure to be used as premises frequently come subsequently to be rejected, and new auxiliaries permitting the derivation of additional observational consequences frequently become available.

Our argument against empirical equivalence now proceeds as follows. As VRO makes clear, the decision to locate a logical consequence of a theory outside its empirical consequence class (on the grounds of the former's nonobsercational status) is subject to change. That class may increase, coming to incorporate an ever greater proportion of the theory's total consequence class (Laudon et al, 2007). This result already shows that findings of empirical equivalence are not reliably projectable, since we cannot reliably anticipate which of a theory's now unobservable consequences maybecome observable. But the problems with empirical equivalence run deeper than the inconstancy of the boundary of the observable. For even if it were possible to circumscribe the range of the observable relative to a state of science, we shall see that it would still be impossible so to circumscribe the range of auxiliary information available for use in deriving observational consequences.

By NAP, a theory's empirical consequence class must be allowed to include statements deducible from the theory only with the help of auxiliaries. One can distinguish the broad from the narrow class of a theory's empirical consequences, where the narrow class contains only observational statements implied by the theory in isolation from other theories and hypotheses. But ANP shows that it is the broad class, containing as Ncell statements deducible only if the theory is conjoined with such auxiliaries, that matters epistemologicalk. Regardless whether holists are right in contending that the narrow class is empty, it is a class of little epistemic moment. It is by the complement of the narrow with respect to the broad that theories are primarily tested, and a characterization of empirical equivalence limited to the narrow would have no such epistemological consequences as we are concerned to contest.

It follows by IAA that, apart from shifts in observational status, a theory's empirical consequence class may increase through augmentations to the theory's total consequence class. As new auxiliary information becomes available, new empirical consequences derived with its help are added. Of course, conditionals connecting the auxiliary statements newly used to the empirical statements newly derived were already present among the theory's logical consequences. But the detached empirical statements are not present until the auxiliaries on which their deducibility depends become available. So long as we include within a theory's empirical consequence class statements derivable from the theory only via auxiliaries, so long as we construe that class broadly - and we have argued that it must be so construed to reflect the realities of theory testing - the theory's logical consequence class will be augmentable in virtue of containing the empirical consequence class as a subset. The empirical consequence class can also diminish, again by IAA, as the rejection of needed auxiliaries discontinues the derivability of some of its members (Pratt et al, 2005). Therefore, any determination of the empirical consequence class of a theory must be relativized to a particular state of science. We infer that empirical equivalence itself must be so relativized, and, accordingly, that any finding of empirical equivalence is both contextual and defensible.

C. Response to anticipated objections

The response we anticipate to our argument is a challenge to its assumption that empirical consequence classes must be identified for their equivalence to be established. Can there not be a general argument to show that classes must be the same independently of determining their membership? An

obvious suggestion is that logically or conceptually equivalent theories must have the same consequence class, whatever that class is. As we do not question the empirical equivalence of logically equivalent theories, we ignore this suggestion and assume henceforth that theories whose empirical equivalence is at issue are logically and conceptually distinct.

One approach to constructing a general argument is to invoke the Lowenheim-Skolem Theorem. This theorem asserts that any firstorder, formal theory that has a model at all has a denumerable model. A standard proof uses terms involving individual constants indexed by the natural numbers as the domain of a model. But if the domain need only be a set of terms, it could just as well be any denumerable set whose members are proposed as the referents of those terms. So, in principle, such a theory has an infinite number of models.

Another approach is to construct an algorithm for generating empirical equivalents to a given physical theory , such as the lowenheim - skolem theorem fails to do for formal theories. For example, there exist instrumentalist algorithms for excising the theoretical terms of a theory without empirical loss. Whether such algorithms are in fact successful is rendered highly dubious by the premises of our argument. It is by no means clear that a theory's instrumentalized version can match its capacity for empirical commitment, once the role of auxiliaries in fixing such commitment and the variability of the range of the observable are acknowledged. At most a theory's instrumentalized version can be held empirically equivalent to it relative to a circumscription of the observable and a presumed or intended domain of application. But while theories fix their own intended interpretations, they do not fix their own domains of application, nor the resources for detection of entities they posit. Algorithmically excised references may pick out entities that become detectable. -New applications may arise with changes in collateral knowledge. Indeed, it is a measure of a theory's success when posited entities acquire a technological role, and applications for which the theory was not designed become possible (Cudd et al, 2003).

The only other approach we know of to establishing empirical equivalence without identifying empirical content is to argue from cases. We propose an example, inspired by van Fraassen's in The Scientific Image, as representative. Let TN be Newtonian theory. Let R be the hypothesis that the center of mass of the expanding universe is at rest in absolute space. Let V be the hypothesis that the center of mass of the universe has constant absolute velocity v. Consider the claim that TN + R is empirically equivalent to TN + V (Fraassen et al, 1997).

This claim is based on the common TN component of the theories. It is Newtonian theory itself that assures us that unaccelerated absolute motion has no empirical consequences of a kind encompassed by the theory; that is, no consequences within mechanics. We can therefore bring two lines of criticism against the claim of empirical equivalence: either there is some other kind of consequence not envisioned within mechanics, or the underlying Newtonian assurance is wrong. The question is whether conceivable developments in scientific knowledge enable us to distinguish the theories empirically on one of these bases.

We can construct an extension of TN+V which agrees with TN+R in not predicting basons . Let TN + W be TN + V plus the hypothesis that there is a velocity w such that basons appear if and only if and to the extent that v>w .

Then the absence of basons establishes only that v does not exceed w ; it does not require R. The presence of besons still refutes TN+R, but TN+R can be supplemented to allow basons ; perhaps they arise spontaneously . TN + R then lacks an explanation of bason production , such as TN + V provides. Something in the way of explanatory parity is achievable by adding to TN + R the hypothesis that what absolute motion produces is antibasons, which immediately annihilate basons. So the presence of basons is explained by the lack of absolute velocity. Still, TN + R does not explain the frequency of bason detection, as TN + V does. The observed frequency must simply be posited, as a constant determined by experiment, and this procedure is an admitted disadvantage relative to TN + V. But this comparison does not affect empirical equivalence.

The appeal to nonmechanical, differentiating phenomena can be defeated, because, if empirical equivalence holds within mechanics, it continues to hold for any extensions of mechanics in which the presence or absence of additional, nonmechanical phenomena is made to depend on the value of a mechanical property. This seems to be a general result. If theories T1 and T2 are equivalent with respect to properties P1, . . . , pn they have equivalent extensions for any enlarged class of proper ties p1 , … , pn , q1, … , qm ; where properties are functions of pl,..., pn. On the other hand, if q1 ,..., qm, are not functions of p they cannot be used to discriminate between T1 and T2 (James, 1996).

3. *II Underdetermination*

We have argued that the thesis that even empirically successful theory has empiricalk equivalent counterparts is precarious, at best. Bu, for

now let us suspend our incredulity about empirical equivalence and suppose that the thesis is sound. We wish to explore in this section what, if anything, then follows from the existence of empirically equivalent theories for general epistemology.

A number of deep epistemic implications, rouehh collectable under the notion of "under-determination," have been alleged for empirical equivalence. For instance, it is typical of recent empiricism to hold that evidence bearing on a theory, however broad and supportive, is impotent to single out that theory for acceptance, because of the availability or possibility of equally supported rivals. Instrumentalists argue that the existence of theoretically noncommittal equivalents for theories positing unobservable entities establishes the epistemic impropriety of deep-structure theorizing, and with it the failure of scientific realism. Some pragmatists infer that only nonepistemic dimensions of appraisal are applicable to theories, and that, accordingly, theory endorsement is not exclusive nor, necessarily, even preferential. One may pick and choose freely among theories whatever works for the problems at hand, so that the distinction between theories and models is lost. In a phrase, the thesis of underdetermination, denying the possibility of adequate evidential warrant tior any theory, has become the epistemic corollary to the presumptively semantic thesis of empirical equivalence (Hacking, 1993).

Against these positions, we shall argue that underdetermination does not in general obtain, not even under conditions of empirical equivalence. As we have seen, empirical equivalence is chiefly seen as a thesis about the semantics of theories; underdetermination, by contrast, is a thesis about the epistemology of theories. It has been supposed that, if theories possess the same empirical consequences, then they will inevitably be equally well (or ill) supported by those instances. We shall contest this supposition and, with it, the reduction of evidential relations to semantic relations, on which it rests. We dispute the ability of semantic considerations to resolve epistemic issues. But even allowing the epistemic dimension we have discerned in empirical equivalence, we shall find that the relative degree of evidential support for theories is not fixed by their empirical equivalence (Hacking, 2003).

A. Evidential results that are not consequences

We begin by noting that instance, of a generalization may evidentially support one another, although they are not consequence, of one another. Previous sightings of black crows support the hypothesis that the next crow to he sighted will be black, although that hypothesis implies nothing about other crows. Supposing this evidential connection to be uncontrocersial, we ask why, then, in the case of universal statements it should be supposed that evidential support is limited to logical consequences. Is it that the evidential connection admitted to hold among singular statements is at best indirect, that it connects those statements only via a general statement that they instantiate? The thesis would then be that direct evidential support for a statement is limited to its logical consequences, and singular statements instantiating the same generalization support one another only in virtue of directly supporting that generalization. In short, where there appears to be evidential support for a statement, s, outside the range of s's logical consequences, such support is parasitic on support of a general statement, m, which entails s, from m's logical consequences (Boyd, 1979).

We believe this to be an unperspicacious way of accounting for what goes on in singular infer-ence. Often the evidential link between singular statements is stronger than the support available for a general intermediary, whose identification can, in any case, prove elusive. But even if this account worked, it should be noted straightaway that allowing a statement to accrue indirect empirical support in this fashion already undermines the claim that statements are confirmable only by their empirical consequences. This result alone suffices to establish that the class of empirical consequences of a statement and the class of its prospective confirming instances are distinct.

We began this discussion with the hackneyed case of black crows in order to show that the possibility of inferences of even the most mundane sort (from particular-to-particular) depend upon demin, the thesis that evidential support accrues to a statement only via its positive instances. This claim becomes even clearer when one considers the manner in which real scientific theories garner empirical support. Consider, for instance, the theory of continental drift. It holds that every region of the earth's surface has occupied both latitudes and longitudes significantly different from those it now occupies.

A number of points are to be noted about such examples. First, by dating them we emphasize that they are not dismissable by invoking auxiliaries via which the evidence is derivable. One could not in the 1890s represent Thomson's results as conse-quences of electrical laws by making electroatomism an auxiliary. Despite Ludwig Boltzmann's pioneering work, statistical mechanics was too speculative in 1905 to qualify as an available auxiliary. Even taking an ahistorical view, it would be casuistical to represent evidence as a consequence of a hypothesis

from which it is derivable via auxiliaries, if it is the auxiliaries rather than the hypothesis that really fuel the derivation. If a formal criterion is wanted, we may stipulate that a hypothesis be ineliminable from the derivation of what are to qualify as its consequences (Boyd, 1979).

Second, the more general theory via which the evidence supports a hypothesis of which it is not a consequence need not be very precise or specific. For example, the statistical mechanics that Brownian motion supported was more a program for interpreting phenomenological thermodynamics probabilistically than a developed theory. There can be good reason to believe that conceptually dissimilar hypotheses are related such that evidence for one supports the other, without possessing a well worked out or independently viable theory that connects them. Perhaps a theory that connected them has been discredited without the connection it effected being discredited. In this respect, nonconsequential evidence for general statements approximates the case of singular statements for which the inferential link proved elusive.

Third, we need not fear running afoul of familiar paradoxes of confirmation in taking evidence to confirm a hypothesis in virtue of supporting a more general statement that implies the hypothesis. The intuition that what increases our confidence in a statement thereby increases our confidence in what that statement entails is fundamentally sound. The difficulties that Carl Hempel, for example, extracted from his "special consequence condition" depend on a certain logical form for general laws and a simplistic criterion of confirmation - Nicod's criterion - which requires, in opposition to the position we have undertaken to defend, that all positive consequences be confirming. Much sophisticated reasoning in the natural sciences would be vitiated by restricting evidence relevant in assessing a theory to the entailments (via auxiliaries) of the theory. And any singular prediction would be so vitiated as well.

Finally, we need to acknowledge and take into account a subtlety of confirmation that might appear to challenge the force of nonconsequential evidence for our argument. There is an obvious way in which a statement not entailed by a theory can be evidence for the theory. The statement might imply another empirical statement that is entailed. Suppose, for example, that the theory entails a - perhaps indefinitely extendable - disjunction of which the statement is a disjunct. By implying a statement that is a consequence, the evidence, though not itself a consequence, fails to discriminate between the theory and any empirically equivalent theory. So showing that there can be evidence for a theory that is not a consequence of the theory does not suffice to show that empirically equivalent theories can be differentially supported.

B. Empirical consequences that are not evidential

Establishing that evidential results need not be consequences is already enough to block the inference from empirical equivalence to underdetermination. But it is instructive to make the converse point as well. Suppose a televangelist recommends regular reading of scripture to induce puberty in young males. As evidence for his hypothesis (H) that such readings are efficacious, he cites a longitudinal study of 1000 males in Lynchburg, Virginia, who from the age of seven years were forced to read scripture for nine Nears. Medical examinations after nine years definitively established that all the subjects were pubescent by age sixteen. The putatively evidential statements supplied by the examinations are positive instances of 1-1. But no one other than a resident of Lynchburg, or the like-minded, is likely to grant that the results support H.

This example has a self-serving aspect. That the televangelist has a pro-attitude toward H on grounds independent of the purported evidence he cites is already enough to make one wary; one need not recognize the flaws in the experimental design of the longitudinal study. In a case without this feature, a person hypothesizes that coffee is effective as a remedy for the common cold, having been convinced by finding that colds dissipate after several days of drinking coffee. The point here is that the very idea of experimental controls arises only because we recognize independently that empirical consequences need not be evidential; we recognize independently the need for additional conditions on evidence (Niven, 1990). No philosopher of science is willing to grant evidential status to a result e with respect to a hypothesis H just because e is a consequence of I{. That is the point of two centuries of debate over such issues as the independence of e, the purpose for which H was introduced, the additional uses to which H may he put, the relation of H to other theories , and so forth .

4. III Formal Constraints on Epistemology

If the identification of empirical consequences with evidential support is so implausible, how has it managed to gain such a foothold? We suggest that a more persuasne, less readily dispelled confusion is ultimately responsible. That confusion, as we have intimated, is to misunderstand the relationship between semantics and epistemology, bringing the largely technical and formal machinery of semantics improperly to bear on epistemic issues.

Specifically, we wish to reveal and challenge the widespread - if usually implicit - conviction that epistemic relations are reducible to semantic relations. It is commonly supposed either that truth and meaning conditions just tire justification conditions, or, at least, that they can be made to double as justification conditions (Niven, 1990). Either way, epistemology is made the poor relation of a family of interconnections among semantic, syntactic, and epistemic concepts, and is left to make do with tools handed down from semantics. It seems to us that distinctively epistemic issues are left unresolved by such a presumed reduction, and that epistemic theses depending on it - such as the underdetermination thesis - are wrongheaded. We will first explain and illustrate the confusion we have diagnosed, then trace the mistaken assimilation of support to empirical consequences to it.

The problem originates in foundationalist epistemology - especially in Descartes' image of a mathematically rigorous, deductive structure for knowledge - and thus is not confined to empiricism. If the evidential relation is deductive, the evidence on which a knowledge claim is based must bear semantic relations to the claim sufficient to permit the deduction.

Perhaps the best modern illustration is the attempt by the logical empiricists to demarcate science by semantic means. Both "verifiability" and "falsifiability," the prime concepts in terms of which scientific status has been delimited, are tools of semantics. Demarcation criteria proposed by the Vienna Circle or its positivist disciples, and by the Popperians, are alike in depending on semantic analysis and syntactic form of statements. What is required for classification as verifiable or falsifiable is basically that a statement satisfy constraints as to logical form and be couchable in observation language. It was assumed on all sides that such conditions suffice to identify the class of statements that are properly the objects of scientific inquiry.

One might think to defend the adequacy of semantic tools for the intended distinction by arguing that the relevant notion of "science" to be demarcated is not that of what passes muster by scientific standards, but merely that of what is up for grabs in scientific inquiry. After all, statements falsified by scientific inquiry are yet to be classed as scientific. But not only is a distinction between what qualifies as scientific and what does not basically epistemic; so too is a distinction between what is worthy of investigation or entertainable by scientific means and what is not. It is basically what we have already found it reasonable to believe that decides these things.

The demarcation problem of the logical empiricists arose as a variant on the logical positivists' program for distinguishing cognitive significance from emotive uses of language misleadingly given propositional form. Already at this level one may discern the assimilation of evidential to semantic relations. For the evaluative force of, e.g., ethical pronouncements that led positivists to disqualify them as genuine propositions is also present in epistemic pronouncements, and, derivatively, in science. Epistemology, it is now commonly recognized, is value- laden (Lycan, 1985). But science was the logical positivists' paradigm of cognitive significance, its propositional status, the ideal to which ethics, religion, and metaphysics futilely spired. If epistemology, and science in particular, was to be salvaged, then epistemic evaluation would have to rest on semantic relations as the only actual alternative to value-free empirical relations.

Of course, this does not make the semantic concept sufficient for the epistemic one, but further developments tended to elevate semantics and syntax over the notions of evidential warrant and rationality of belief used in other truth conditions for knowledge attributions. The incompleteness of the list of truth conditions was manifested in a curious asymmetry between the truth and evidence conditions. If the truth condition is not met, no bolstering of the evidence is sufficient for knowing. But inconclusive evidence that leaves open the possibility of error can be sufficient for knowing, if only, as a matter of fact (or happenstance), the world cooperates. In many celebrated paradigms of knowing, the evidence needed does not seem all that strong (Lycan, 1985). Thus, attention focused more on the truth condition than the evidence condition - more on semantic than epistemic issues. Ironicallc, the recent emergence of reliability theory, which re-emphasizes the justificatory component of knowledge in the tradition of Gettier's challenge, underscores the paucity and defeasibility of the evidence on which ordinary knowledge relies. Add to this asymmetry the success of Tarski's theory of truth in contrast to the sorry state of theories of evidential warrant, and one has the makings of a semantic and syntactic orientation for epistemology.

Given this orientation, it was natural to approach the problem of warranting a hypothesis - the problem of testing-by attending to statements that bear syntactic and semantic relations to the hypothesis - to its instantiations. At least this was natural for empirical generalizations, whose instantiations are empirical statements. This approach then created so many internal problems and tasks - Hempel's paradoxes of confirmation across logical

relations; Goodman's problem of projectibility - that the possibility of warrant provided by statements syntactically and semantically independent of the hypothesis was lost sight of. Instantiations of theoretical hypotheses are not empirical, but an assimilation of support to consequences was somehow extrapolated for them, by supposing them in principle recastable in observational terms or, perhaps, by supposing their testability reducible to the testability of empirical generalizations. Such was the hold of the resulting picture, that the assimilation of support to consequences exceeded the confines of logical empiricism to capture the format of textbook characterizations of scientific method itself. Although written by a philosopher, Hempel (Landau, 2006) the following passage will strike every reader as stereotypical of standard accounts of empirical inquiry:

5. Results

Results that test a theory and results that are obtiainable as empirical consequences of the theory constitute partially nonoverlapping sets . being an empirical consequence of a theory is neither necessary nor sufficient to qualify a statement as providing evidential support for the theory . because of this , it is illegitimate to infer from the empirical equivalence of theories that they will fare equally in the face of any possible or conceivable evidence (Laudan, 2005). The thesis of underdetermination , at last in so far as it is founded on presumptions about the possibility of empirical equivalence for theories – or " systems of the world " – stands refuted .This ubiquitous assimilation of a theory's test cases to its logical consequences in an observation language, as we have argued above, wrongly ignores some of the more salient ways of testing theories. Worse, it generously greases the slide from empirical equivalence to underdetermination and epistemic parity. Ironically, the limitation of a statement's justification conditions to its truth conditions represents a striking break with the traditional empiricist project. Prior to the emergence of neopositivism in the 1920s, the general idea about theory testing and evaluation was that there was a range of "phenomena" for which any theory in a particular field was epistemically accountable. (In planetary astronomy, for example, these phenomena would be observations of positions of the planets, sun, and moon.) A theory's success or failure was measured against these phenomena, and decided by the theory's ability to give an account of them. A theory was, of course, responsible for its entailments, but it was held equally accountable for all the relevant, established phenomena, and could not evade this responsibility by failing to address them. For a Newton, a Ptolemy, or a Mach, "saving the phenomena" meant being able to explain all the salient facts in the relevant domain (Leplin, 1909). With the rise of neopositivism, the epistemic responsibilities of theories were radically reinterpreted. Theories became liable only for what they entailed. Failure to address relevant phenomena, or at least to be indirectly applicable to them, now emerges as a cheap way of protecting such success as a theory does achieve, rather than as a liability. Where empirical adequacy formerly meant the ability to explain and predict all the salient phenomena, it now requires only possession of none but true empirical consequences. Recall the passage lately quoted from van Fraassen. The radical character of the shift we are describing becomes immediately clear there when one notes his identification of "empirical adequacy," saying only true things about observable features of the world, and "saving the phenomena." Prior to our time, no one would have supposed, as does van Fraassen, that saving the phenomena amounts only to possessing an observable model. No one would have supposed, as does van Fraassen, that a theory is to be judged only against the correctness of its own observational commitments (be those commitments expressed in model-theoretic or propositional form), irrespective of the comprehensiveness of the class of such commitments, irrespective of the theory's applicability to problems independently raised. It is testimony to the pervasiveness of the thesis that epistemic assessment is reducible to semantics that van Fraassen's conflation of the hitherto quite disparate notions of empirical adequacy and saving the phenomena has gone unnoted.Much epistemology in our day is arbitrarily and unreasonably constrained by these developments. Our concluding, positive moral is that epistemic warrant unfettered by semantics has rich and varied sources yet to be exploited.

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The evaluation of different levels of Manta pulagum on performance, and blood parameters of broilers

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Abstract: This study was conducted to investigate the effects of using different levels of Menta pulagum on performance, carcass traits, blood biochemical and immunity parameters of broilers. At first 300 one day old broiler chicks were divided to 5 groups and five replicates of 12 chicks in each group. Experimental groups included T1, control group with no Menta pulagum supplementation, T2, T3, T4, and T5 received 0.75%, 1%, 1.5%, and 2% Menta pulagum respectively. The results showed that the use of different levels of Menta pulagum has significant effects on performance and carcass traits of broilers ($P<0.05$). The highest level of weight gain was in group 5 also the highest percent of liver and breast were observed in group 5 but the greatest percent of thigh was observed in group 4. The results evidence that the using of Menta pulagum in broiler feeds have significantly effects on performance and blood biochemical and carcass traits without having any significantly effects on immunity parameters except the level of heterophile to lymphocyte.
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Keywords: Blood parameter, Broiler, Performance, Menta pulagum

1. Introduction

The first usage of antibiotics in poultry science goes back to the 1946. By scientific demonstration of antibiotic advantages in livestock, the usage of these syntetic products increased for many years. After many years, the long term side effects of these products like microbial resistance and increase of the blood cholestrol level in the livestock lead to the ban of these commercial antibiotics. Nowaday, there are a lot of concerns to finding non-synthetic alternatives for antibiotics among the scientists. The positive effect of herbal plants on broilers have been reported by many studies (Tekeli, 2006). Unfortunately, over use of these products ended up with a lot of problems both for animals and costumers, for example, bacterial resistance to antimicrobial agents (Javed, 2006). Because of this problem, there have been made some restricted rules about the usage of these antibiotics, like ban and low use of them (Kamel, 2001).

Organic poultry is a relatively new expression in western countries which is going to expand in other countries. In this kind of poultry method, farmers do not use chemical compounds at all or in a very low level for sake of costumers, instead they use alternatives like organic acids, probiotics, and medicinal plants, and despite of higher prize of this method, these products have more fans in the costumers (Ipu, 2006). There are a lot of reports indicating the positive effects of herbs like anti-coccidal , anti-oxidant, anti-fungi and etc. Some of medical effects of herbs are related to their secondary metabolites such as phenols, necessary oils, saponins and etc (Ipu, 2006). Herbs have been used for some disease since long time ago because of availability, easy usage, non side effects. Many herbs have a long history of use even prehistoric use, in preventing or treating human and animal diseases. Aromatic plants have been used traditionally in therapy of some diseases worldwide for a long time. Research on the use of herbal mixtures in broiler diets has produced inconsistent results (Fritz, 1993). Some authors state significant positive effects on broiler performance (Ertas, Cross, Peric, 2005, 2007,2008), whereas another group of authors established no influence on gain, feed intake or feed conversion (Cross, Ocak, 2007, 2008).

The objective of this study was to investigate the effects of using different levels of Menta pulagum on performance, carcass traits, blood biochemical and immunity parameters of broilers.

2. Material and Methods

In this experiment that started 1 day following until 42 days that there are four treatments, at first 300 one day old broiler chicks were divided to 20 groups of 15 chicks each. Each 5 groups randomly assigned to one of the 5 treatments. control group with no thyme supplement, T2, T3, T4, and T5 received 0.75% ,1%, 1.5%, and 2% thyme powder respectively. There were similar partition for male and female birds into treatment groups. The rations were similarly formulated in all treatment groups based on the NRC, 1994 Nutrients recommendations (tables 1).

Dried Menta pulagum was supplied from local market and after fine milling, mixed with other ingredients. The diets and water was provided *ad libitum*. The lighting program during the experiment period consisted of a period of 23 hours light and 1 hour of darkness. Environmental temperature was gradually decreased from 33 ° C to 25 ° C on day 21 and was then kept constant.

Performance parameters

During days 0-42, unbound water and dietary was in poultries' access. Dietary and chick weigh were going on weekly. Feed consumed was recorded daily, the uneaten discarded, and feed conversion ratio (FCR) was calculated (total feed: total gain). At the end of experiment, some analyses was done via SAS (SAS, 2001). (Statistical Analyses Software) in the statistical level of 5% according to data gathered from dietary, average of FCR.

Carcass traits

At 42 days of age, four birds per replicate were randomly chosen, slaughtered and carcass percent to live weight and percent of carcass parts to carcass weight were calculated.

Immunity system:

In the 35th day of experiment, three chicks were chosen from each group and inoculated from brachial vien by 0.1 ml (5%). Heterophils to Lymphocytes ratio were determined and Globulin and Albumin proportion in blood were counted from blood samples which had been obtained from barchial vein of three randomly chosen chicks from each group in the 49th day of experiment.

Serum parameters:

Blood samples were obtained from barchial vein and centrifuged in order to getting serum, after 12 hours of fasting in the 49th day of experiment. Serums have been analyzed for glucose, Cholestrol, Low-density lipoprotein (LDL), High-density lipoprotein (HDL) and Triglyceride by ELISA set.

Statistical analysis:

After obtaining the data, they were analyzed by variance method (ANOVA) considering ($P < 0.05$) using SPSS 18 software. The significant differences were taken to Duncan multiple range test to compare the means.

The result from above figures is that the structure in frequency band of 0-70 is of 4 peak of displacements. The significant point is that the maximum amount of displacement is exactly created in principle frequency places. As figures show maximum amounts are in frequencies of 10.431 and 24.588 which are exactly equal to principle frequencies amounts from modal analyses and it is a reason for accuracy of modal analyses.

Table 1. Ingredients and chemical analyses composition of the starter and grower diets

Ingredients (g/kg)	1-28	29-42
Maize	557	300
Wheat	--	330
Soybean meal	370	300
Soybean oil	30	40
Fish meal	20	--
Limestone	10	--
Oyster shell	--	12
Dicalciumphosphate	5	15
Vitamin-mineral mix[2]	5	5
dl-methionine	1	1
Sodium chloride	2	2
Vitamin E (mg/kg)		--
100		
Zn	--	50
Analyzed chemical composition (g/kg)		
Dry matter	892.2	893.5
Crude protein	222.3	200.7
Fat		62.4
62.9		
Fiber	36.1	35.6
Ash	61.7	57.0
Calcium	8.22	8.15
Phosphorus	5.48	5.57
Selenium (mg/kg)	0.53	0.58
ME by calculation (MJ/kg)	12.78	12.91

[1] starter diet fed to birds from 0 to 21 days. [2]Provides per kilogram of diet: vitamin A, 9,000 IU; vitamin D3, 2,000, IU; vitamin E, 18 IU; vitamin B1, 1.8 mg; vitamin B2, 6.6 mg B2,; vitamin B3, 10 mg; vitamin B5, 30 mg; vitamin B6, 3.0 mg; vitamin B9, 1 mg; vitamin B12, 1.5 mg; vitamin K3, 2 mg; vitamin H2, 0.01 mg; folic acid, 0.21 mg; nicotinic acid, 0.65 mg; biotin, 0.14 mg; choline chloride, 500 mg; Fe, 50 mg; Mn, 100 mg; Cu, 10 mg; Zn, 85 mg; I, 1 mg; Se, 0.2 mg.

3. Result and Discussion

The effects of different levels of Menta pulagum on performance of broilers are showed in Table 2.Using different levels Menta pulagum have significant effects on weight gain and feed conversion of broilers but there was not significant effect on feed intake.

The effects of different levels of Menta pulagum on carcass traits of broilers are in Table 3. Application of different levels of Menta pulagum significantly affected the carcass traits ($P<0.05$). The highest percent of liver was observed in group 5. There are a possibility of gathering these to antimicrobial herbs made a remarkable decrease in

the amount of intestine microbal colony and this prevented from lysis of amino acids and they used in formation of proteinic tissues and increased the breast percentage. *Lee et al* (Lee, 2003) found that the existence of harmful microbes in digestive system causes an increase in the lysis of protein and amino acids of nutrients, di-amination activity of proteins and amino acids and rapid decomposition of these molecules due to secretary substances from bacteria like urease. Considering this fact and antimicrobial activity of these herbs, the whole matter seems sensible.

The present of antioxidants and phenolic substance in Menta pulagum may be the main cause of improvement in breast percent of broilers carcass. The presence of harmful bacterial populations in the gastrointestinal tract may cause breakdown of amino acids and thereby reduce their absorption as antimicrobial substances are present in thyme can reduce the harmful bacterial populations in the gastrointestinal tract and improve the levels of absorbed amino acids (Lee, Zargari, 2003,2001). The carvacrol in Menta pulagum has stimulatory effects on pancreatic secretions by increasing the secretions of digestive enzymes more amounts of nutrients like amino acids can be digested and absorbed from the digestive tract and thereby improve carcass traits. Else increasing the percents of gizzard and liver by positive effects via physically grinding and increasing bile secretion on nutrient digestion. With increased amounts of absorbed amino acids, organs like breast and thigh drawn more growth.

The effects of different levels of Menta pulagum in starter and grower feeds on blood biochemical and immunity parameters of broilers are summarized in Table 4 and 5.

The use of different levels of thyme did not have any significant effects on immunity parameters of broilers except Heterophils to Lymphocytes ratio. The mean values of serum constituents in broiler chicken fed different supplemented diets are shown in table 5.The serum total cholesterol, Triglycerides and LDL concentration were significantly reduced in group of 5 compared to the control group ($P < 0.05$). The concentration of serum HDL and Glucose were not significantly effects in compared to the control group.

The main reason of cholesterol and triglycerids decrease in blood of chicks is substances like carvacrol and tymol which are present in herbs. These substances have effect on cholesterol and triglyceride and decrease these harmful parameters in blood (Zargari, 2001). According to *Akiba* and *Matsumoto* high level of fibers can increase the excretion of bile and this can decrease the cholesterol level of blood (Lee, 2003). Since these plants have high level of fibers so this can one of other influences of carvacrol is on immune system, it can improve immune system of chickens. The rate of heterophile to lymphocyte is an important index in evaluating immune system, the higher rate of this ratio shows that immune system has been weakened and an increase in the body inflammation (Sturkie, 1995).

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Table 2: Effects of treatments on performance of broilers

Treatment	Weight gain (gram/day)	Feed Intake (gram/day)	Feed Conversion
T1	38.28 [a]	78.21 [a]	2.11 [a]
T2	37.62[a]	78.11 [a]	2.21 [a]
T3	39.32 [b]	79.29.5 [ab]	2.10 [b]
T4	39.15 [b]	81.62 [c]	2.16 [b]
T5	43.61[ab]	81.79[c]	2.28[b]

[a-c]Means with different subscripts in the same column differ significantly ($P < 0.05$)

Table 3. The effect of different levels of Menta pulagum on carcass traits of broilers

Parameters	T1	T2	Treatments T3	T4	T5	SEM
Abdominal Fat	3.77[a]	3.82[a]	3.52[a]	3.41[a]	3.43[a]	0.35
Gizzard	3.19[a]	3.20[a]	3.22[a]	3.36[a]	3.54[a]	0.29
Breast	32.08[b]	33.02[b]	34.63[ab]	34.45[ab]	35.20[ab]	0.76
Thigh	26[a]	25.48[a]	25.42[a]	28.63[ab]	27.37[ab]	0.52
Liver	3.07[a]	2.93[a]	3.25[a]	4.01[ab]	4.32[ab]	0.21

[a-c]Means with different subscripts in the same column differ significantly ($P < 0.05$)

Table 4: Effect of treatments on immunity system of broilers

Parameters	T1	T2	T3	T4	T5	SEM
Heterophils to Lymphocytes ratio	0.27	0.28	0.29	0.25	0.19[ab]	0.24
Globulin	1.46	1.64	1.44	1.52	1.69	0.44
Albumin	1.38	1.42	1.52	1.50	1.64	0.21

Blood Parameters	Parameters	T1	T2	Treatments T3	T5	SEM
Glucose (mg/dl)	Abdominal Fat	3.77[a]	3.82[a]	3.52[a]	3.43[a]	0.35
Cholesterol (mg/dl)	Gizzard	3.19[a]	3.20[a]	3.22[a]	3.54[a]	0.29
HDL	Liver	3.07[a]	2.93[a]	3.25[a]	4.32[ab]	0.21

[a-c]Means with different subscripts in the same column differ significantly ($P < 0.05$)

Table5. The effect of different levels of Menta pulagum on blood biochemical of broilers

Blood Parameters	T1	T2	Treatments T3	T4	T5	SEM
Glucose (mg/dl)	172.55[a]	170.89[a]	173.82[a]	174.19[a]	173.35[a]	18.29
Cholesterol (mg/dl)	135.35[b]	133.77[b]	133.82[b]	128.54[ab]	126.19[ab]	21.25
Triglyceride (mg/dl)	42.37[a]	40.69[a]	39.45[a]	36.22[b]	33.95[b]	4.21
LDL	33.19[a]	34.23[a]	32.96[a]	30.28[b]	30.25[b]	1.29
HDL	79.54[b]	79.21[b]	82.42[b]	82.89[b]	83.11[b]	1.61

[a-c]Means with different subscripts in the same column differ significantly (P < 0.05)

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Evaluating Competition of the *Phalaric minor* in Wheat

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Abstract: to competitive effects of wheat and *p minor* in densities and different use values of Nitrogen in plan frame of random blocks repeating 4 times perfectly. The test factorials included Nitrogen value in level (100,150 and 225 kg/ha) and *p minor* density in 5 levels (0, 20, 40, 80 and 160 bones per square meter). Results of the test showed that height of *p minor* per three values of Nitrogen was less in initial processes of growth and more than wheat in final processes of growth. Increasing Nitrogen value has caused to increase leaf and biomass of *p minor* and increasing *p minor* density causes area of leaf and biomass of wheat to decrease. Time of closing canopy in *p minor* is more rapidly than in wheat. The most value of decreasing operation of wheat seed was about 48 percent while was observed in density of 160 bones of *p minor* in 225 kg/ha of Nitrogen. Average relative growth velocities of wheat and *p minor* were 0.073 and 0.028 g/dag during growth cycle, respectively. Little use value of Nitrogen fertilizer, reason of more decrease of wheat operation was existence of *p minor* grass.
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Keywords: Wheat, *p minor*, Competition, Nitrogen, Density

1. INTRODUCTION

Phalaric minor exists in most of Iran provinces as a result of consistency with different conditions of biology and ecology; also, it is a most common species of grass. In modern management of grasses, emphasis is on management of grass societies instead of try to cancel them which needs modernized relations of grasses with agro plant to be known a curtsy. Understand relations of agro plant and grasses for management to be used in long-term needs competition in view of grasses to be considered (Mortimer et al. 1997). Competitive effects of grasses on a product would be affected with the both species (Carlson et al. 1985). Plants are in competition for resources like light, water and (mineral) nutrients. Such resources are distributed among plants that require them crucially.

As there is a linear relation between biomass generating and absorption of growth limiting resources, biomass is reflector plant of limited distributed among them. According to variable distribution of dry material in plants bodies with competition stresses, inter competition in plants throughout measuring total biomass. Competition is a complicated event that in under influencing by different biologic, environmental and vicinity factors. According to relation of operation, density and present resources, plant density is an important factor in study of competition. Effect value of grasses on wheat operation depends on different factors like kind and density of grass bone, agro type, and value and time period of used fertilizer, date and rows and other ecological conditions.

For example, *wild oat* and *Brassica Kaber* are better than wheat in receiving soil Nitrogen while in optimum conditions of agriculture; these two grasses are more effective in decreasing operation of this agro plant as a result of wider growth (Henson et al. 1982). In samples associated with *wild oat*, when its density was %106 green cover, increase of neither Nitrogen fertilizer nor cause to increase in wheat operation of course caused it to be decreased notably(Carlson et al. 1985). Agriculture with small type in compare with high type one increases Spike, seed product and dry weight of *wild oat* about 80-100 (Torner et al. 1986). Studies in Khuzestan showed when density of *A. Ludoviciana* was about 70 bones per square meter; wheat operation was decreased to %66. Competition of *A.Ludoviciana* and wheat is variously depending on product type and grass density (Attalian et al. 2003). According to this fact that competition of grass and agro plant differs influencing by ecological conditions. Among food materials, Nitrogen is the one that follows worry relating with competition of grasses; many researches about effect of Nitrogen on competition of agro plants with grasses. Increasing Nitrogen fertilizer in wheat associated with *wild oat* causes grass density to me increased and agro plant operation to be decreased (Carlson et al. 1986). Increasing Nitrogen value in rice was in favor of *Cyprus rotundus* and caused right absorption, leaf area index and rice operation to be decreased together (Okafor et al. 1976). *Chenopodium album* and *polygonum convolvulus* show better reaction in higher levels (Haas et al. 1982). *Brassica kaber* was increased

while increasing Nitrogen in soil from 20 to 120 mg/kg in soil and responded more than wheat (Iqbal et al. 1997). In spite of increasing operation of agro product due to fertilizer use without competition conditions, on the other hand causes density and biomass of grasses to be increased too which it may be followed with increasing seed product and since it can have had a positive correlation with biomass, so fertilizer uses such as Nitrogen will be affective on seed product (Fawcett et al. 1978). this research was done to consider competitive reaction of wheat with variation value of used fertilizer of Nitrogen and density of *p minor* to appoint competition effects on biological and economical operations of wheat; in fact wheat response to Nitrogen fertilizer ready for *p minor* competition for competitive effects of this grass.

2. materials and methods

This research was done in 2010 agro year in Islamic Azad University, Agriculture college, including two factors: Nitrogen use value in three levels (N1=100, N2=150 and N3=225 kg/ha) and density of *p minor* in five levels (0, 20, 40, 80and160bones per square meter).

This research was done in 4 random blocks plan frames of factorial type. Kind of used wheat name d CHAMRAN was applied with density of 450 bones per square meter by seed system distancing 17 cm of the considered one. Phosphorus fertilizer was used 150 kg/ha with seed. This agro operation was done associating with using 1/3 fertilizer in each processes of operation. Width of each field scene was considered as 3m and distance between each two scenes was considered as 2m canceling effects among different Treatments. Length of each scene was 6m with 2m distance between each two blocks (reputation). There were 5 bones per scene while past agro was vegetables type. Soil sampling showed existence of 21.6ppm mineral Nitrogen to 60cm depth in the field soil. Rows of *p minor* were between each two separate rows of wheat while each level had a distance 20cm of the other row and 10cm with the nearest wheat one. Break of rest (sleep) for *p minor* was done with making them wet for 20 hours in water and then compactness with Gibberellins acid (350 ppm). Other required watering was done according to plant needs in season. All of grasses except *p minor* were cancelled by 2, 4-D herbicide system and manually. Doing the research, there was no illness and death. To consider growth trend from the last of wheat preparing, measurements of height, leaf area and dry weight of each sample were done after random taking wheat plant and *p minor* from level of 0.2 square meters. After closing canopy, general distribution of level on samples was measured in 5 levels with 25cm in thickness (0-25, 25-50, 50-75, 75-100,100-125). Analysis of data and drawing diagrams needed were done by using sigma plot, MSTATC and Excel and to compare averages, Dankan multi domain test in probability level 5 percent was used.

3. Result and discussion

A. Wheat height and p minor

The height of species was influenced by value of Nitrogen fertilizer while varying used Nitrogen fertilizer from 100 to 150 kg/ha caused to increase the wheat height and increasing from 150 to 225 kg/ha caused it to increase height of *p minor*. Increasing density of *p minor* didn't affect on wheat height to 40 bones on *p minor* to 80 bones, so after densities of 40 and 80 bones of *p minor* a notably increasing in height. In seems that planting of *p minor* happens sooner that wheat and it limits space development for wheat has more capability to absorb Nitrogen around wheat bone through bones interacting (Martin et al. 1998). And as a result of being height due to desirable use of *p minor*, it takes 95 days after planting more rather than wheat that height increasing happen till the end of growth season. Height has an important role in competition process so that increasing Nitrogen from 100 to 225 kg/ha, height of wheat decreased from 103.18 to 105.8 cm (Table1). Often being higher the wheat, height of *p minor* was more improved than wheat so that its final height was 12-14 cm more than wheat (Cudney et al. 1989). Interaction of light absorption and photosynthesis in wheat is as a result of big height of *p minor*, that is shown using competition of *p minor* with wheat in the last of capability season of competition.

TABEL I. Compare of averages of main effects on different areas in competition of P. minor

Timours	Height(cm)		Leaf Area Index	
N	wheat	p. minor	wheat	p. minor
100	92.81 a	103.18 b	3.6a	0.35 b
150	90.5b	102.12b	3.6 a	0.49 c
225	88.37c	105.8a	3.4b	0.62 a
p. minor D(p/m2)				
0	88.6 c		3.8a	
20	89.1c	101.7b	3.8a	0.15 d
40	89.3c	102.1b	3.6b	0.26 c
80	91.3b	102.7b	3.3c	0.5 b
160	94.7a	104a	3.09d	0.91 a

In each column means followed by similar letters are not significantly different (p=%5)

B. Index of leaf area

This index was under influence of value of Nitrogen fertilizer used both species of wheat and *p*

minor. Considering data of the index showed that variation of value of Nitrogen fertilizer from 100 to 225 kg/ha has been caused the above index to be decreased from 3.6 to 3.4 for wheat (Table1). On the other hand, density of *p minor* with increasing from 0 to 1610 bones per square meter caused the index of wheat to be decreased from 3.81 to 3.9(Table 1). Statistical analysis of the data in 145 days after planting having maximum of the leaf area showed that increasing the Nitrogen used would increase the index of leaf area of *p minor* (Table1) and also density of *p minor* increased from 0.15 to 0.91 (Table1). increase of density due to decrease of nutrient value particularly and increase of shadowing causes oldness and leaf falling particularly down of canopy which it will be followed in decreasing area of leaf of wheat (Iqbal et al. 1997). Distribution of leaf area of the species showed that *p minor* grass has the most density of leaf area in 50-75 cm layer and wheat in 25-50 cm one(Hasanzade et al. 2000). Increasing *p minor* from 20 to 160 bones was associated with decrease in index of wheat leaf area and increase in index of *p minor* leaf area has known index of leaf area as the best criteria of expressing the capability of photosynthesis and authority for growth of each species (Potter et al. 1977). Considering relative share showed that percentage of leaf area of *p minor* was very little rather then wheat and was 26 percent of leaf area of canopy related to *p minor* in the highest level of the *p minor* of 160 bones per square meter. with and wheat in different values of used Nitrogen, index of leaf area for Wild oat is less than wheat, since that competition capability of *p minor* was reported more than wheat and it was said that further photosynthesis level of each species, there are other effective factors in competition capability like distributing leaves inter canopy (Cudney et al. 1989). So height, index of leaf area and vertical distribution of leaves inter canopy through competition process (Kropff et al. 1981). Expressed that the suitable criteria for evaluation of decrease value of agro plant operation is a close relation between decrease of plant operation and ratio of leaf area of grass. It was observed that the highest level of index of *p minor* leaf area in about through 145 days after field working and the most index of leaf area wheat CHAMRAN kind about 160 days of it expressing that *p minor* canopy is closed easier than wheat. It was expressed that time of closing the canopy of agro plant and grass is an important in competition process and plant which reaches this stage, has higher competition capability (Cousense et al. 1991). Comparing distribution of leaf area shows that wheat has the most index of leaf area in 25-50 cm layer and *p minor* in 50-75 cm layer. So use value of Nitrogen fertilizer is effective on distribution of wheat leaf area. So that is concentrated in use value of 100kg Nitrogen per hare with maximum index of leaf area in 25-50 cm layer that increase of Nitrogen fertilizer, index of leaf area of wheat increases in 50-75 cm layer. On the other hand, general distribution of leaf area index in *p minor* is concentrated in middle of canopy rather than the other layers. The most of index of leaf area for *p minor* in dependent on environmental conditions is concentrated in upper half of canopy *p minor* having more index of leaf area to the upper layers, has more capability rather than wheat for reaction with high competition. The speed of being yellow for leaves of down layers of wheat was more than wild oat (Beyschlag et al. 1990). In *p minor*, Nitrogen consumption was increased from minimum value to maximum one in index of leaf area for the upper layers, that seems Nitrogen consumption causes index of leaf area transport to the upper layers through increasing height and inter nodes.

C. Speed of products growth

Increasing density of *p minor* and speed of *p minor* growth will be less with little value of Nitrogen rather than higher level and speed of *p minor* growth has been in ceased with increase to 225kg Nitrogen per hear (Figs. 1, 2). Increase of *p minor* density causes decrease of leaf area of wheat, so speed of wheat growth will decrease.

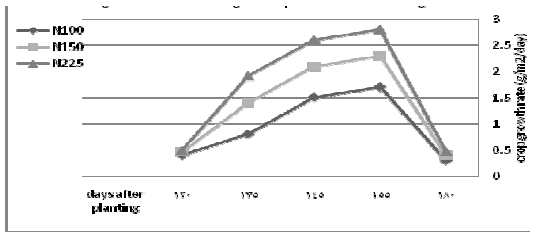

Figure 1. Variation of wheat growth speed in different nitrogen

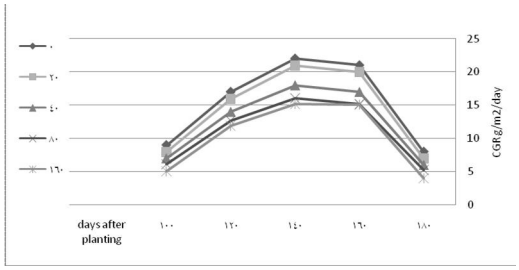

Figure 2. Wheat CGR changes in different P. minor density

D. Speed of relation growth

Speed of relative growth for is more than it for wheat. Speed of relative growth for *p minor* was very low at first and it was less than it for wheat too. But as a result of suddenly increase of leaf area of *p minor*, speed of relative growth increased after a period of 95-115 days from harvesting. Speed of relative growth decreases for wheat and grass of *p minor*. Speed of relative growth was lees than wheat in growing season early but increased after that rather than wheat (Cousense et al. 1991). Nitrogen increase caused speed of relative growth of *p minor* to increase. Increase of *p minor* density affected on speed of relative growth for wheat. When height of *p minor* is less than it for wheat in growth early, but it will be more using Nitrogen fertilizer and better competition conditions, so *p minor* will shade on canopy of wheat and its speed of relative growth will effect on wheat.

E.Economic operation

Economic operation was effected by *p minor* density, Increase of density to 80 and 160 bones per square meter caused wheat operation to decrease. This value was 341 kg/ha for increase of density from 40 to 160 bones of *p minor*. That is, wheat operation decreases when increase of bones per square meter and this value will be 2.8 kg/ha. If *p minor* would not be controlled with 20 per square mater value till product season (Caussanel et al. 1993), decrease of wheat operation will be 8.7-21.8 percent. Decrease of wheat operation was 33.9 to 54.2 percent (Salimi et al. 1996). Economic operation comparison of wheat rather than Timor without *p minor* in the less and most values of Nitrogen use showed that wheat operation decreases when *p minor* density increases. So that in the less value of Nitrogen, decrease of operation was 29 percent when *p minor* density increased to 160 bones per square meter whiles it was 37 percent when increase value of Nitrogen was 160 bones per square meter in *p minor* density. This comparison showed that competition power of *p minor* increases when Nitrogen and *p minor* density increase. Have known wheat operation decrease in competing wild oat due to decrease of cluster number (Angonin et al. 1996). Competition of *p minor* decrease seed number of wheat in up levels of grass density and up levels of Nitrogen. Simultaneously of quick growth rate for *p minor* height and wheat sprayings, applied shading of leaves causes wheat seed number and economic operation to decrease. A given trend was observed for effect of *p minor* density and Nitrogen effect on harvest index. Nitrogen value affects on wheat index with up level density of grass. Increase of Nitrogen caused wheat index to decrease. So decrease of operation was more than before it when *p minor* density and Nitrogen increase.

4. CONCLUSION

Wheat operation expresses inter species competition of this grass in upper levels (Cousens et al. 1985). Application of upper values for Nitrogen fertilizer cause wheat product per area unit as a result of its given effect on biomass and created leaf area per area unit. Also it was recognized that lower densities of *p minor* with less values of Nitrogen causes economic operation to more values. According to growing ability and much shading with long height for *p minor* and seeds produce, managing this grass through less density and seeds bank per area unit causes this grass to decrease; unavoidable use of chemical fertilizers should be done in base of pollution values of *p minor* and gullibility of seeds bank when pollution of this grass reaches to up levels.

At first, pollution was decreased using management affairs and then chemical fertilizer should be used with desired values.

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Heavy Metals Enrichement in Deposited Particulate Matter at Abu Zaabal Industrial Area –Egypt

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Abstract: This study aims to assessment the impact of two major polluted industries in Egypt located in Abu Zaabal industrial area. Deposited particulate matter was monitored at Abu Zaabal industrial area around Awadalla lead smelter and abu Zaabal phosphorus fertilizer plant. This study also provides information on the distribution of trace elements in dust fall during different times and areas. Dust fall was collected monthly from November 2008 to October 2010. Heavy metals were extracted by nitric acid and measured using Perkin Elmer 6100 ICP/MS. Dust fall (DF) results revealed that the annual mean rate of deposited dust collected during the year 2009 was 29.49, 33.54, 35.64, and 36.83 g/m^2.30days for sites no. 1, 2, 3 and 4 respectively while during the year 2010, the annual mean rate of deposited dust was 28.53, 34.40, 164.46 and 295.65 g/m^2.30 days for sites no. 1, 2, 5 and 6, respectively. Sites no 5 and 6 recorded higher P_2O_5 concentration and deposition rate than sites 1 and 2 that is due to the impact of fertilizer plant. High enrichment factors for heavy metals (Pb, Cd, and Ni) were found in dust-fall samples, while Zn, Co and Cr showed minimal to moderate enrichment.

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Key words: Industrial area, Deposited particulate matter, Heavy metals.

1. Introduction:

Quality of life is a balance between technology progress and environmental risks. This balance can be controlled by analytical chemistry, which is in a central position. Elaboration and rapid determination of anthropogenically toxic elements is urgently required **(Rahmalan *et al.*, 1996)**. Many scientists believe that current concentrations of pollution-derived particulate matter (PM) in ambient air are deadly causing thousands of premature deaths annually **(Colburn and Johnson, 2003; Dockery *et al.*, 1993; Dominici *et al.*, 2003; Kjellstrom *et al.*, 2002; Netherlands Aerosol Programme, 2002; Pope *et al.*, 2002; Samet *et al.*, 2000; Schwartz, 1991; U.S. EPA, 1997)**. Such premature deaths are thought to be due to both long-term and short-term exposure to ambient PM. Human exposure to trace metals occurs primarily through inhalation of air and ingestion of food and water. It was estimated that more than 15 10^9 kg of particulate matter from anthropogenic sources are emitted into the air each year **(EPA ,1976)**. Abu Zaabal industrial area is located in the north of Cairo city and comprise more than 700 industrial facilities beside Abu Zaabal for special chemicals and Abu Zaabal for fertilizers and chemicals industries. Awdalla lead smelters was transferred to Abu Zaabal since 2000 beside railways workshops.

The primary objective of this work is to put into perspective some of the current concepts with respect to trace metals distribution and emissions in air. This will reflect the potential adverse on health effect due to human exposure to trace metals.

2. Material and Methods

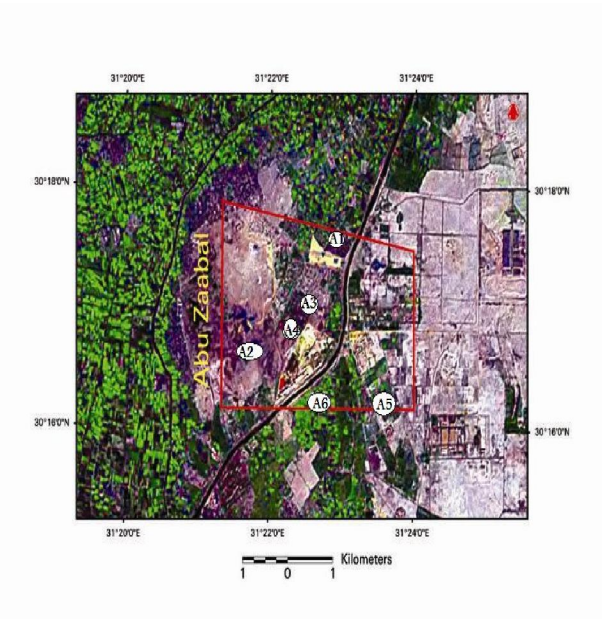
Dust fall is monitored using the American Society for Testing and Materials standard method for collection of dust fall – **ASTM Method D1739**. Deposited particulates matter were collected monthly for two successive years from Nov. 2008 to October 2010 and dust fall collectors were used for collecting dustfall as previously used in Egypt. **(Ibrahim, 2000)** .The collector consists of cylindrical glass beakers 17 cm height and 8 to 9 cm diameter. The cylindrical glass beaker was mounted on a height of 150 cm above roof level to prevent dust from disturbances close by to have an impact. Dust fall jars were replaced at the beginning of each month and were investigated for heavy metals content. Jar content was transformed quantitatively carefully to a dry, clean weighed beaker using successive washing with distilled water till the inside of the jar become clean. Successive dry and weighing the beaker were made until constant weight, the differences in weight represent the amount of deposits dust during the corresponding month at each site of the present study. For heavy metals determination and P_2O_5 in deposited dust, method 3050B **EPA Standard Methods** was used for the digestion of samples by weighing definite weight of sample then digested with repeated additions of nitric acid (HNO) and hydrogen peroxide, hydrochloric acid (HCl) was added to the initial digestate and the sample was

refluxed then filtrated and rinsed with hot HCl and concentrated by heating then completed to 25 ml volume flask and measured by inductively coupled plasma (ICP) but fluoride ion was determined by using selective ion electrode.

Table (1): Dust fall sites in Abu Zaabal industrial area

Sites NO.	UTM Coordinates		
		Northing	Easting
1	North of lead smelter	30172967	312255.42
2	Beside lead smelter	301642.50	312144.92
3	Central of Abu Zaabal	301707.10	312235.16
4	Railways hospital	301700.84	312214.22
5	200m Eastern of Fertilizer plant	301527.96	312224.16
6	100m Eastern of Fertilizer plant	301606.13	312211.75

Fig. (1): Coordination map of Abu Zaabal studied area



3. Results and Discussion:

Tables (2 and 3) summaries the annual mean rate of deposited dust collected during the year 2009, it was 29.49, 33.54, 35.64, and 36.83 g/m^2.30days in Abu Zaabal industrial area for sites no. 1, 2, 3 and 4 respectively while during the year 2010, the annual mean rate of deposited dust was 28.53, 34.40, 164.46 and 295.65 g/m^2.30 days for sites 1, 2, 5 and 6, respectively. Site no. 5 and site no. 6 was located in the Eastern side of Abu Zaabal Fertilizer plant .received higher deposition rate of particulates than other sites that is due to the impact of fertilizer plant on the surround area. Fugitive dust and stake emission especially during charging and discharging of rock mills are the main sources of particulate emissions. The rate deposition of dust is considered 4 and 8 times more than the rate of deposition recorded at shoubra El-Khima industrial area where rate deposition ranged from 39.5 to 44.6 **(Ibrahim, 2000)**. This rate of deposition is considered very heavy deposition according to Pennsylvania guidelines for dustfall **(Stern, 1986)**, moreover, these rates of deposition exceed in terrible amount of standards for dust deposits in many countries ,for instance, the air quality standards in USA is 5.7 g/m^2.30 days and is 1.93 g/m^2.30 days as a background value **(Stern, 1986)**. While it is 14 g/m^2.30 days for industrial areas according to Egyptian law 470 / (1971). **The Environmental Information and Monitoring Program of Egypt (2011)** reported that, western countries normally state that whenever dust fall values are less than10 g/m^2 per 30 days, the area may be considered clean and according to **SANS (2005)**, the new dust fall standard has been established and widely used in determination the potential of dust fall in residential and industrial areas. **SANS (2005)** standard threshold stated that deposited dust in industrial area lies between 18 and 36 g/m^2/30days (Table 6) so that only sites no. 5 and 6 show the vigorous increase of dust fall than other sites and also exceeding the alert limit to take action of 72 g/m^2/30days to immediate action and remediation required following the first incidence of dust fall rate being exceeded. Fig. (2 and 3) refer to seasonal variation of deposited dust rate in Abu Zaabal industrial area during years 2009 and 2010. Although the deposition rate is higher in sites no. 5 and 6 than other sites but the seasonal variation stile refers to the increase of deposition rate during winter than other seasons while summer season shows the lowest deposition rate during years 2009 and 2010.This finding can be attributed to several factors, the most important factors are lack of rain fall in summer which washout the dust particulate from atmosphere**.**

Heavy metals concentrations in dust fall:

Lead smelters were transferred from Shobra El-khima since 2000 to El- Safa desert in Abu Zaabal, beside railways workshops and Abu Zaabal phosphorus fertilizers plant. One of the objects of the current study is to evaluate the concentration of lead metal and companied heavy metals after transferring

the lead smelters to Abu Zaabal. table (2 and 3) show the increment of Pb in site no. 2 beside lead secondary smelter than other sites .it can be noticed that lead and nickel levels (1215, 994 µg/g respectively) are much higher than the levels recorded at sites no. 1,3,4,5 and 6 , this is may be due to the factories which emit high concentration of metals to surrounded area and threaten the quality of life to those people live in this area. Lead level recorded in the present study was considered very high when compared to other cities for example in Amman, Jordan where lead level recorded was 74µg/g **(Kamal, 2000)** this means that Abu Zaabal lead level was more than 10 times the lead level in Amman but in Shobra El-Khima the level of lead was much higher than the level of lead in Abu Zaabal. Ni level in Shoubra El-Kima was 230µg/g **(Ibrahim, 2000)** this value was about 20% of Ni deposited over Abu Zaabal (table2). Chromium, zinc, cadmium, cobalt recorded the highest values at sites no. 5 and 6 located beside the Abu Zaabal fertilizer plant, which ensure the impact of this factory in contaminating the surrounding area with heavy metals .The concentrations of those elements were much high higher than the concentration found at site no. 1 which located upwind to sites no. 5 and 6. The annual mean concentrations of P_2O_5 during the year 2009 were 38.45 µg/g, 45.18 µg/g, 48.85 µg/g, recorded in sites no. 2, 3 and 4, respectively ,while the concentrations recorded for P_2O_5 in sites no. 2, 5 and 6 during the year 2010 were 34.26 µg/g, 84.6 µg/g, and 158.3 µg/g, respectively, those values are significantly higher than P_2O_5 level in site no. 1 .Site no. 1 located upwind to the investigated area and away from the impact of lead smelters and fertilizer plant. The annual mean concentration of hydrogen fluoride present in dust fall during the year 2009 was higher at sites no. 5 and 6 than that recorded at site no.1 (0.23µg/g) , while sites no. 5 and 6 recorded 7.06 and 8.98 µg/g (table2). This data clearly show the vigorously impact of Abu Zaabal fertilizer plant on the surrounded area.

Generally the impact of Abu Zaabal phosphorus fertilizer plant is responsible of increasing the of heavy metals, HF and P_2O_5 concentrations in deposited dust over the surrounding area. This finding is clearly confirmed by the values recorded for site no.1 (tables 2,3). The maximum concentration of HF and P_2O_5 recorded during the study period were 158.3 and 8.98 µg/g respectively, those results considered terrible when compared with the same results recorded at site1.

Table (2): Annual average of deposited dust (g/m^2.30days) and some trace elements (µg/g) in Abu Zaabal industrial area during the year 2009.

2009		1	2	3	4
DF	Mean	29.49	33.54	35.64	36.83
	SD	2.69	4.22	4.65	4.13
Cr	Mean	16.23	37.33	26.09	30.82
	SD	4.71	6.19	9.20	8.94
Ni	Mean	59.18	994	415	468
	SD	15.80	134.71	129.99	140.17
Pb	Mean	51.72	1215	242	316
	SD	19.95	234.	112	144
Zn	Mean	133.43	311.52	175.47	209.39
	SD	27.08	90.67	33.71	47.29
Cd	Mean	2.32	6.68	3.95	4.52
	SD	0.69	2.13	0.92	0.77
Co	Mean	14.49	28.18	19.97	21.54
	SD	3.45	6.50	2.24	2.05
HF	Mean	0.38	2.52	2.37	3.05
	SD	0.19	0.83	0.83	0.93
P2O5	Mean	0.41	38.46	45.13	48.86
	SD	0.20	11.24	19.48	18.80

Table (3): Annual average of deposited dust (g/m^2.30days) and some trace elements (µg/g) in Abu Zaabal industrial area during theyear 2010.

2010		1	2	5	6
DF	Mean	28.53	34.40	164.46	295.65
	SD	1.86	4.91	19.35	43.67
Cr	Mean	13.27	29.57	46.57	69.84
	SD	4.61	6.90	13.42	17.70
Ni	Mean	47.45	907.5	526.1	787.4
	SD	19.65	219.1	159.9	211.1
Pb	Mean	38.82	1102.	335.7	497.5
	SD	9.67	284.6	117.5	142.2
Zn	Mean	105.8	399.3	522.9	786.4
	SD	30.91	93.54	157.6	196.7
Cd	Mean	2.13	5.08	11.65	16.69
	SD	0.61	1.59	3.19	2.87
Co	Mean	13.78	26.20	35.54	53.30
	SD	2.83	5.78	8.93	11.26
HF	Mean	0.23	2.07	7.06	8.98
	SD	0.15	1.00	2.26	2.31
P2O5	Mean	0.53	34.27	84.61	158.3
	SD	0.27	11.10	16.73	24.46

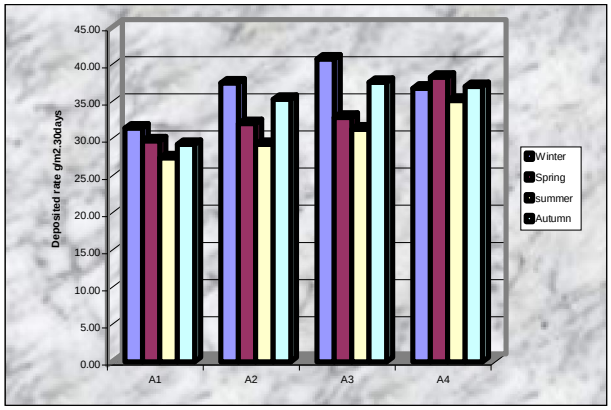


Fig. (2): Seasonal variation of deposition rate of Abu Zabal industrial 2009

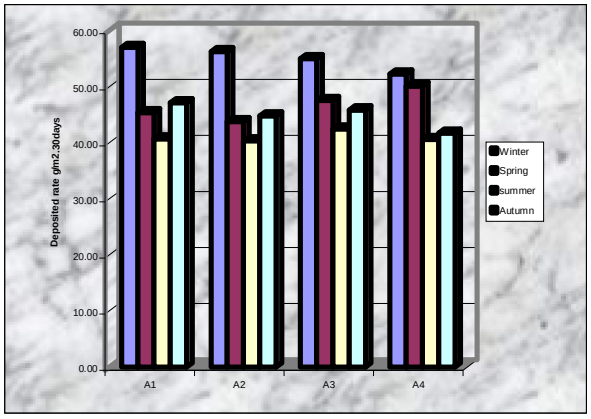


Fig. (3): Seasonal variation of deposition rate of Abu Zabal industrial 2010

Table (4): Correlation coefficient of duat fall and heavy metals in Abu Zaabal during the year 2009

	Cr	Ni	Pb	Zn	Cd	Co
Cr	1.00					
Ni	0.96	1.00				
Pb	0.86	0.96	1.00			
Zn	0.94	0.99	0.98	1.00		
Cd	0.98	1.00	0.95	0.99	1.00	
Co	0.98	1.00	0.94	0.98	1.00	1.00

Table (5): Correlation coefficient of duat fall and heavy metals in Abu Zaabal during the year 2010

	Cr	Ni	Pb	Zn	Cd	Co
Cr	1.00					
Ni	0.60	1.00				
Pb	0.15	0.88	1.00			
Zn	0.99	0.72	0.30	1.00		
Cd	0.99	0.51	0.04	0.96	1.00	
Co	1.00	0.63	0.19	0.99	0.98	1.00

Table (6): Air quality management guidelines for Dust Deposition – SANS 1929:2005

Band Number Band description	**Label**	**Dustfall rate (D) (g/m^2/30day)**	**Action taken**
1	Residential	D<18	Permissible for residential and light commercial
2	Industrial	24 < 0 < 36	Permissible for heavy commercial and Industrial
3	Action	36 < D < 72	Requires Investigation and remediation if two sequential months lie in this band, or more than three occur in a year.
4	Alert	72 < *D*	Immediate action and remediation required following the first incidence of dust fall rate being exceeded, Incident report to be submitted to relevant authority.

Determination of correlation coefficient:

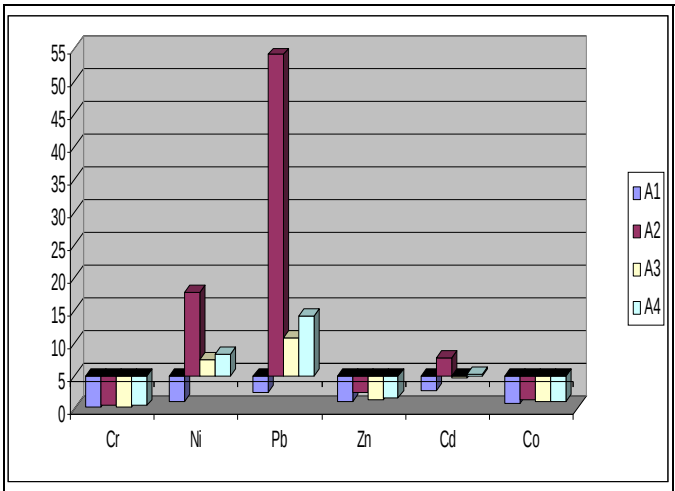
The correlation coefficient. between the heavy metals in dust falls at Abu Zaabal were studied . The correlation coefficients were evaluated as an index of dependency among metals and dust fall (DF). Tables (4&5) show a strong positive correlation between Cr, Ni, Zn, Cd, and Co this strong correlation between elements refers to probability of originating from the same origin. It can be concluded that heavy metals is a contaminants of fugitive dust emited from Abu Zaabal fertilizer plant and also emphasise the role of this plant in contaminating Abu zaabal area.

Determination of enrichment factor (EF) in dust fall:

To evaluate the magnitude of contaminants in the deposited dust, EF were computed for each location relative to the abundances of species in source materials to the control/background value and the following equation EF= (C_m/C_{Al}) sample/ (C_m/C_{Al}) control/background value was used Where (C_m/C_{Al})

sample is the ratio of concentration of trace metal (C_m) to the concentration of Al (C_{Al}) in the deposited dust samples and (C_m/C_{Al}) control/background value is the reference ratio in the control/background value. Al is selected as reference element because, it is mainly supplied from sediments and it is one of the widely used reference elements **(Sekabira *et al*., 2010)**. Cr is minimal enriched in all sites of abu zaabal during the years 2009 and 2010,cobalt also was minimally enriched in deposited dust except at site 2 during the year 2009 and sites 5 and 6 during the year 2010.

Ni, Zn and Cd are significantly enriched in site no. 2, located beside lead smelters area, the power of enrichment increase as the distance from smelter decrease that is why those elements are considered to be originated from industrial activities in Abu Zaabal ,while Pb, Ni, and Cd significantly enriched in sites 3 and 4. while pb was extremely high enriched in site no. 2 during the study period (2009 and 2010.). Generally, heavy metals in Abu Zaabal dust fall show the significant enrichment of Ni, Pb and Cd while other metals was minimal and moderate during the years2009 and 2010.



Fig(4): Enriched Heavy metals in Abu Zaabal dust fall during 2009 show the significant enrichment of Ni, Pb and Cd while other metals was minimal and moderate

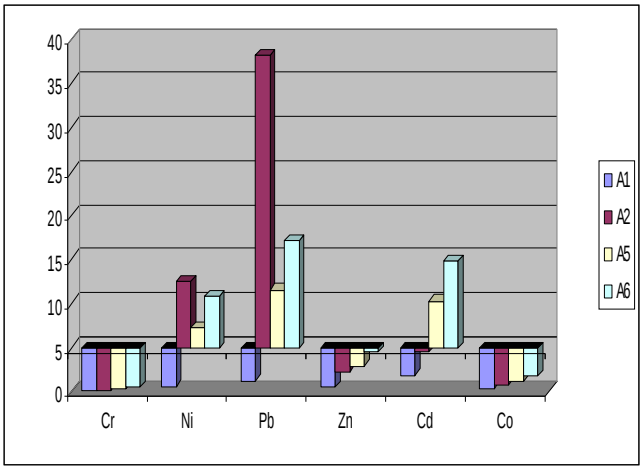


Fig (5): Enriched Heavy metals in Abu Zaabal dust fall during 2010 2009 show the significant enrichment of Ni, Pb and Cd while other metals was minimal and moderate

Conclusion

Lead smelters increased not only the concentration of lead in deposited dust but also increased other toxic heavy metals (Cd, Pb, Ni and Zn) in area near to lead smelters. Rate of deposited dust was exceeded the permissible limit of Europe But according to SANS new standard and alert limit only sites no. 5 and 6 exceeded the alert limit and need action to start mitigation in studied area. Pb , Ni and Cd were significantly enriched in dust fall during he years 2009 and 2010. Phosphurs fertilizer plant emit high amount of particulate matter contaminated with heavy metals beside HF and P_2O_5 , so that this factory has a big role in contaminating the surrounding area. Focusing and environmental consciousness must be taken into consideration in certain heavy metal.

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Polyamide 6.6 planed Polyester: A New Prosthetic Fabric for Repair of Superficial Digital Flexor Tendon Deficit in Equine

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Abstract: A new prosthetic material polyamide 6.6 planed polyester was used for repair of lacerated and cut digital flexor tendons in equine especially those accompanied by deficits and gap formation. This work was done experimentally on 17 donkeys and applied on 5 clinical cases .The prosthetic material was placed and fixed between the two cut ends to reconstruct the tendinious defect. Clinical, ultrasonographic, histopathologic and biomechanical evaluations were performed to judge the efficiency of the prosthetic implant. Satisfactory results were obtained regarding healing of the affected tendons, the return of the animals to their normal ambulation and gait. Clinical cases showed good healing in extensor tendon lacerations and superficial digital flexor cuts. Complications were encountered in a case of deep digital flexor cut with large gap. It was found that, the new Polyamide 6.6\ Polyester fabric (1:1) proved to be strong and biocompatible in addition its low cost in comparison with other prosthetic material.

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Keywords: prosthetic material; polyamide; tendon; equine; Polyester; fabric

1. Introduction:

Tendon lacerations are very common in equine especially in the lower limbs. These injuries represent a difficult issue for equine practitioners due to the extended length of time required for healing and the high rate of recurrence (Davis and Smith; 2006 and Dyson;2004).

Repair of equine flexor tendon lacerations are usually managed primary by suturing through using large monofilament material or by second intention by immobilizing the affected limb and allowing the wound to heal**.**

Tendon graft included autogenous graft and synthetic graft using different synthetic fabrics (Jann *et al.*;1992 and Valdés-Vazequez *et al.*; 1996).The use of such augmentation devices help in bridging any gap that may develop after tenorrhaphy and thus serves as a scaffold and a source for fibroblasts migration and increasing the presence of dense tendon fiber bundles (El-Husseiny, 2000). Natural carbon fibers have been used by many researchers with various results (Weiss et al., 1985)**.** The formation of sinuses accompanying the use of carbon fibers limited its use especially if the risk of infection was considered (El-Husseiny, 2000).

Nylon (polyamide 6.6) is a synthetic material which proved considerable results when used for tenorrhaphy in equine limbs. It has minimal tissue reaction, low incidence of bacterial infection and can be used in contaminated wounds (Jann *et al.*;1992) where polyamide has many characteristics as high biological properties, ability to decrease absorption of fluids, smoothness, flexibility and high resistance to rot (Tan *et al.*, 2003 and Raghavendra *et al.*, 2004). Braided polyester fibers proved to have a high breaking strength (Leknes *et al.*; 2005)**,** resistance to crease, high tensile strength, high abrasion resistance and high capacity to resist micro-organisms, insects, body compatibility (Booth; 1975 and Gupta;1998). In addition to the woven fabric techniques produce from polyamide\ polyester is characterized by Dimensional stability, Fineness and Durability (Gupta;1998).

The aim of the present work was to evaluate the efficiency of a new synthetic mesh (Polyamide 6.6\ Polyester 1:1) for the repair of severed digital flexor tendons in equine.

2. Materials and methods

The present study was carried out on 17 donkeys (experimental animals) and 5 horses (clinical cases).

1- The experimental animals were divided into 2 groups:

- **Control group**: included 5 donkeys with apparently healthy limbs intended for biomechanical studying of the tensile strength of the normal superficial flexor tendons (SDFT).
- **Gap defect group:** included 12 apparently healthy donkeys intended for surgical interventions by implantation of synthetic Polyamide 6.6: Polyester fabric. They were

divided into four equal groups (3 donkeys for each group) according to the time of observation and follow up at 15, 30, 60 and 90 days postoperatively.

Prosthetic material:

Polyamide 6.6\polyester (50 %: 50%): a new mixture of synthetic fibers (Fig. 1) was used to insheath of experimentally transected tendons. The material was available in the form of sheet with tissue density of 1.14gm/cm³ for the polyamide and 1.38 gm/cm³ for the polyester and specifications of woven fabric polyamide\polyester in (El-Husseiny, *et al.;* 2011). This material was sterilized by autoclaving at 121°C for 30 minutes before use according to the melting point for polyester (180ºC)and polyamide (263ºC) (Booth;1975).

Anaesthesia and control :

All operations were performed under the effect of anaesthetic regimen composed of Acepromazine/Xylazine (o.1, 1.1mg/kg respectively) as premedication and Induction was done by Ketamine Hcl (2.2 mg / kg) while maintenance was done by intravenous injection of Thiopental sodium 5% (5mg/kg).

All animals were controlled in lateral recumbency with the operated limb uppermost.

The surgical technique:

A 5 cm skin incision was done at the mid metatarsus of the right hind limb. Following dissection of the sub cut tissues, the paratendon was incised and the SDFT was exposed, exteriorized and transected then about 2 cm of its tendinious tissue was removed. An appropriate piece of the material was wrapped in the form of a cylinder to insheath the two cut ends of the SDF tendon. The material was sutured in position with interrupted stitches using polydioxanone (PDS) suture material No. 1. Paratenon was sutured by simple continuous suture pattern using PDS suture material No. 2/0 then the skin was sutured by simple interrupted suture pattern using PDS suture material No. 1 (Fig, 2 and 3).

Postoperative care:

A series of casts with windows and therapeutic shoes were applied to gradually increase the load placed on the repaired tendon. The casts were applied on the distal part of the limb with the fetlock flexed to form a dorsal angle of approximately 190° that decreased to 170° after 15 days according to (Valdés-Vazequez**;**1996)**.** Hand walking was commenced after the second cast had been removed. All operated animals received a systemic course of antibiotic (Cefotaxime (ETHICON.PROD.CO), 25mg/kg given intramuscular.

Postoperative follow up :

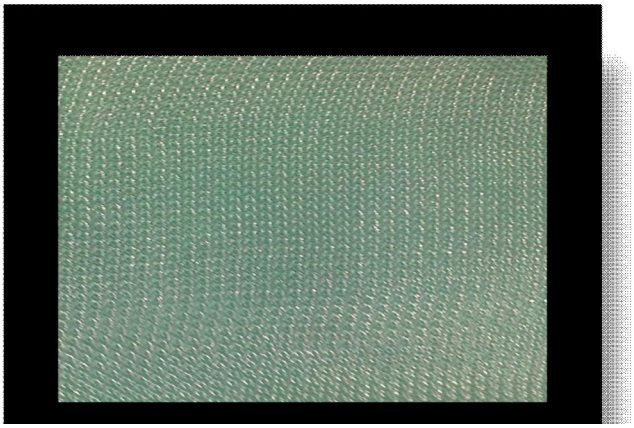
Clinical, ultrasonographic, biomechanical and histopathological evaluations were performed to all operated animals.

- **The clinical evaluation** included daily observation of the seat of operation for any signs of infection or rejection, weight bearing capacity and full limb function.

-The ultrasonographic evaluation included sequential ultrasonographic examination at 15,30,60 and 90 days postoperatively using a high frequency linear probe (8- 10 MHz, TOSHIBA (JUST VISION 200))

- **The biomechanical evaluation** was carried out at the Textile Division– National Research Center in Cairo; the woven sample synthesized from \polyester materials with plain 1/1 structure, using monofilament yarn, the sample was used raw without treatment.

Physical and mechanical tests before implant (El-Husseiny, *et al.;*2011) and after implant were carried out on the sample after conditioning the fabric for 24 hours under the standard atmospheric conditions (20±2°C temperature, 65 ± 2% relative humidity). using a tensile testing machine (Instron 3345®, England) accordance with (ASTM D5035). This was done after euthanasia of the control and experimental animals and collection of the tendon specimen. In the control group, A 20 cm length sample was taken from the SDF tendon at the mid metacarpal area. In the operated group, the samples included the reconstructed tissue and the host- graft interface. Samples were taken at the end of each respective observation period (15, 30, 60 and 90 days postoperatively) and tested for both the tensile strength and strain.



Fig, 1: Polyamide 6.6: polyester fabric in the form of woven fabric.

Fig, 2: An appropriate piece was wrapped around the two cut ends of the SDFT.

Fig, 3: The material was sutured in position with interrupted stitches using PDS suture material.

- **Histopathological evaluation:** Gross and histopathological examinations were performed through harvesting the implantation site to document the visibility and stability of the proximal and distal host- graft interfaces grossly. The evaluation was completed through histopathological examination according to (Bancroft *et al.*; (1996)**.**

2- The clinical study:

The clinical study was performed by application of the new synthetic implant fabric on 5 horses admitted to the surgery clinic, faculty of veterinary medicine, Cairo University. Two cases were admitted with complete cuts in the long digital extensor tendons and 2 cases suffered from complete cuts in the SDFT. One case showed complete cut in both the SDFT and DDFT.

3. Results

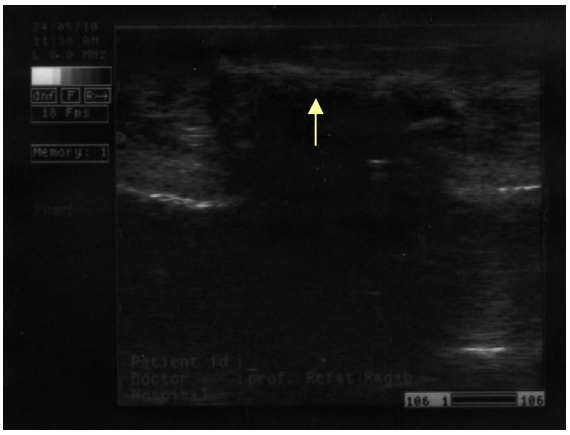
The new prosthetic material (polyamide 6.6: polyester) was used successfully for repair of 12 cases of experimentally transected SDFT. The success of this material was judged according to the following evaluations:

Clinical evaluation:

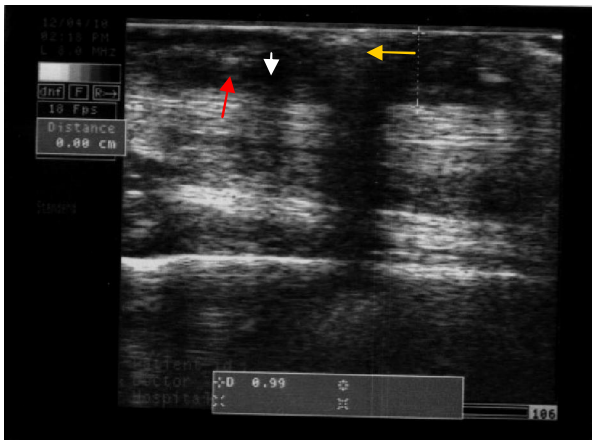
- **7 days postoperatively**: an intense inflammatory reaction was observed in the form of swelling, hotness and pain on palpation.
- **15 days postoperatively**: the skin wound was completely healed with a marked swelling at the implantation site and a clear gap was detected by palpation. The fetlock angle didn't show any palmar deviation and all operated animals were able to use their operated limbs in slow movement.
- **30 days postoperatively**: the local postsurgical inflammatory reaction was subsided and the gap between the two cut ends of the tendon was replaced by soft tissue.
- **90 days postoperatively**: the gap could not be detected and palpation of the tendon revealed homogenous, uniform structure connecting the two cut ends with difficulty in discrimination of the implant from the tendineous structure. All animals were able to use their limbs effectively in slow and fast movements.

Ultrasonographic evaluation:

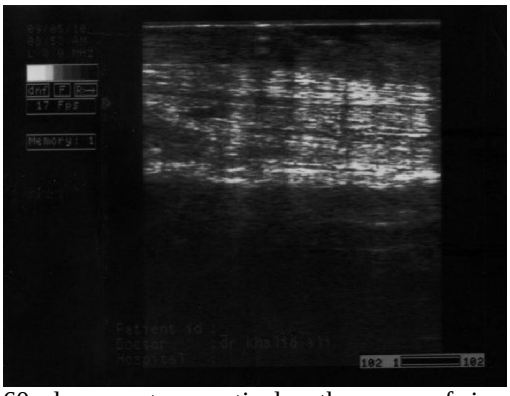
-**15 days postoperatively**: the implant appeared as a thick hyperechoic band connecting the hypoechoic two cut ends of the tendon with a clear distal acoustic shadowing. Anechoic to hypoechoic areas were also noticed representing the inflammatory reaction (Fig.4).
- **30 days postoperatively**: the hyperechoic structure representing the implant was reduced while a hypoechoic structure representing the early fibrosis and granulation tissue formation was increased (Fig.5).
- **60 days post operatively**: the implant material was difficult to be distinguished with minimal distal acoustic shadowing. The area of implantation appeared homogenously hypoechoic with detection of parallel echogenic lines representing tenofibrilis that occupied about 50% of the whole thickness of the tendon (Fig, 6).
- **90 days postoperatively**: complete union between the implant and the two cut ends of the tendon .The operated SDF attained its normal ultrasonographic appearance except for the presence of distal acoustic shadowing representing the remnants of the implant (Fig. 7) .



Fig, 4:15 post operatively, the implant appeared as hyperechoic structure connecting the two cut ends of the SDFT with clear distal acoustic shadowing (arrows).



Fig, 5: 30 days post operatively, the area of implantation showed mixed echogenicity; hyper echoic areas with representing the implant material (yellow arrow), hypoechoic areas representing the early fibrosis and granulation tissue formation (red arrow) and anechoic areas reflecting the inflammatory edema (arrow head).



Fig, 6: 60 days post operatively, the area of implantation appeared homogenously hypoechoic with parallel echogenic lines representing the tenofibrilis occupying 50% of the whole thickness of the tendon.

Fig, 7: 90 days post operatively, showed complete union between the implant and the two cut ends of the tendon except small hyperechoic area with distal acoustic shadowing representing the remnant of the implant (arrow).

Biomechanical evaluation: :

The values of the tensile strength and strain for both control and operated samples at the end of the respective observation periods, 15, 30, 60 and 90 days postoperatively were collected in table (1):

Table(1): Biomechanical evaluation of the normal (control) and operated SDFT.

Name of sample	Time Implant	Tensile Strength (KN)	Strain (Mm)
SDFT (control)	Zero Time	1625.89 ± 20	5.529±0.7
Polyamide 6.6: Polyester implant (gap defect group)	15 days	149.033±15	1.4±0.25
	30 days	725.688±20	2.4±0.312
	60 days	980.66±15	3.2±0.245
	90 days	1504.364±30	4.4±0.521

It was observed that, 15 days postoperatively, the tensile strength was 149.033±15 KN compared with that of the normal control group 1625.89± 20 KN. This value increased in the operated group during the time of healing to reach 1504.364±30 at the end of the experiment (90 days

postoperatively) which was nearly equal the normal value of the control group. The strain of the healed tissue represented 1.4± 0.25 mm at the early stage, 15 days postoperatively, compared with that of the normal control (5.529±01mm) which increased by time to reach 4.4 ±0.521 mm at the end of the experiment ,90 days postoperatively, which was nearly equal to the normal control value.

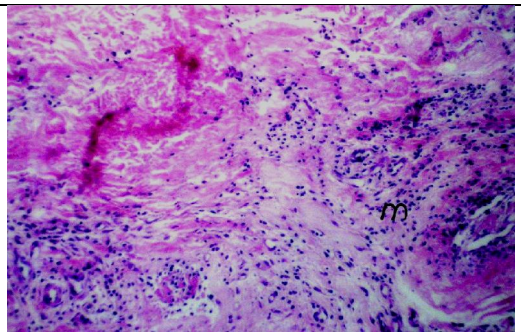
Gross and histopathological evaluation:

Macroscopically, 15 days postoperatively, the implant was clearly distinguished from the two cut ends of the tendon but fusion between the implant and the original tissue was good. Intense inflammatory reaction was recognized in the form of redness of the proximal and distal tissue-graft interfaces. Microscopically, granulation tissue in the form of newly formed capillaries and granulation tissue proliferation was observed associated with inflammatory cell infiltration (Fig.8 &9).

30 days postoperatively: the implant was difficult to be distinguished and the repaired tissue became more homogenous. The inflammatory reaction was diminished as represented by mild redness allover the implantation site. Microscopically, inflammatory cell infiltration was detected associated with fibrosis and granulation tissue formation. The predominant cells were fibroblasts along with a smaller number of macrophages and mast cells indicative of active matrix synthesis of collagen and a tissue of a relatively significant strength filled the tendon defects (Fig 10 & 11).

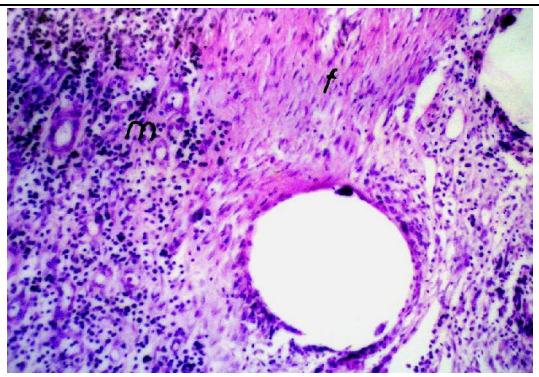
90 days postoperatively: specimen appeared completely mature, the implant could not be distinguished as it became covered by tendineous tissue. The tendon resumed its normal gross appearance and became uniform and homogenous. Microscopically, few focal cell infiltrations were observed associated with remodeling of the tendineous fibers and realignment of the fibers into a longitudinal pattern of parallel collagen fibers. At this stage there were remnants of the implanted fabric surrounded by mature fibrous connective tissues and little macrophages (Fig.12&13).

Fig, 8: Two weeks post operatively, the implanted material between the two cut ends of the tendon (red arrow) with intense inflammatory reaction in the form of redness of the tissue graft interface (yellow arrow).



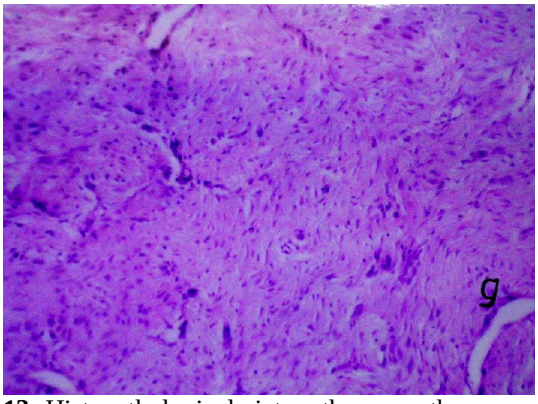
Fig, 9: Two weeks post operatively, showing inflammatory cell infiltration and congested blood vessels**(H and E × 100)**

Fig, 10: 30 days post operatively, showed pink tissue (arrow) bridging the gap between the transected tendon ends. Suture material had remained interatendiniously and the prosthetic material was difficult to be distinguished.



Fig, 11: Histopathological picture one month post operatively, showing area of inflammatory cells infiltration (m) with granulation tissue formation (f) **(H and E × 100)**.

Fig 12: 90 days postoperatively, showing complete union between the graft and original tendineous.



Fig, 13: Histopathological picture three months post operatively, showing area of healing with granulation tissue (g) **(H and E × 100)**.

The clinical study:

The application of the new prosthesis revealed good results in the 2 cases suffered from complete long digital extensor cuts, manifested by marked improvement in the progression that occurred 15 days post implantation, (Fig. 14a, b, c & d).

Cases suffered from complete SDF cuts (2 cases) showed satisfactory results regarding the wound healing and the limb function. One month post surgery, animals used their limbs soundly in slow movements and returned to their normal progression.

One case in which there was cuts in both SDF and the deep digital flexor tendons failed and didn't show any improvement. The case was admitted with a large tendineous defect (10 cm gap) and massive skin loss. The case was considered of bad prognosis as infection and massive granulation tissue was formed and the animal could not use its limb effectively (Fig, 15).

Fig, 14a: Two year old male horse with complete laceration of the long digital extensor tendon. .Note, the presence of wound at the dorsal aspect of the metatarsus with marked flexion of the phalangeal joints due to absence of extensor tendon function.

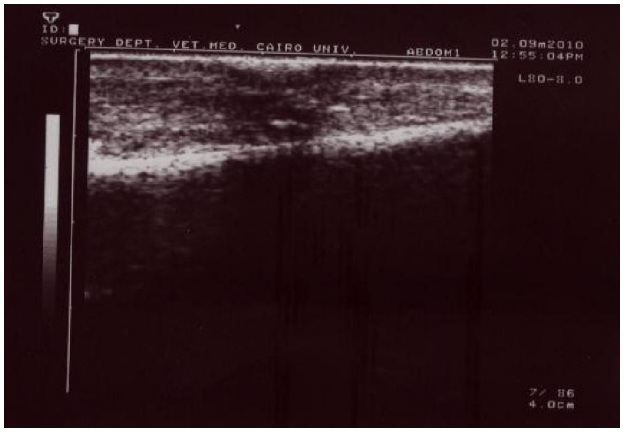


Fig. 14b: Longitudinal sonogram of the same case showed complete cut of the long digital extensor (LDE) tendon. Anechoic area (arrow) between the two cut ends representing the site of tendon cut.

Fig, 14c: One week post treatment, showing the wound healing with absence of infection

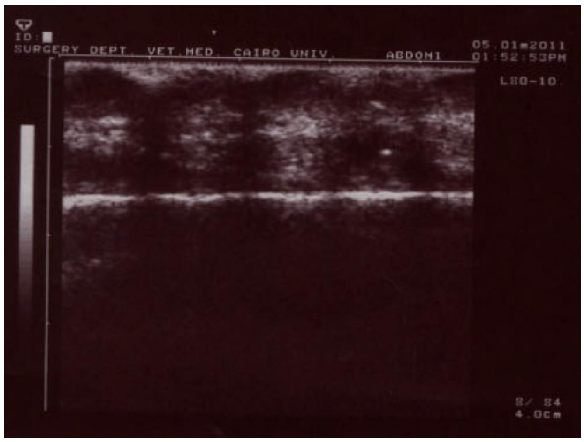


Fig14d: Ultrasonographic follow up showing the implant material as hyperechoic structure with distal acoustic shadowing between the two cut ends of the tendon (arrows).

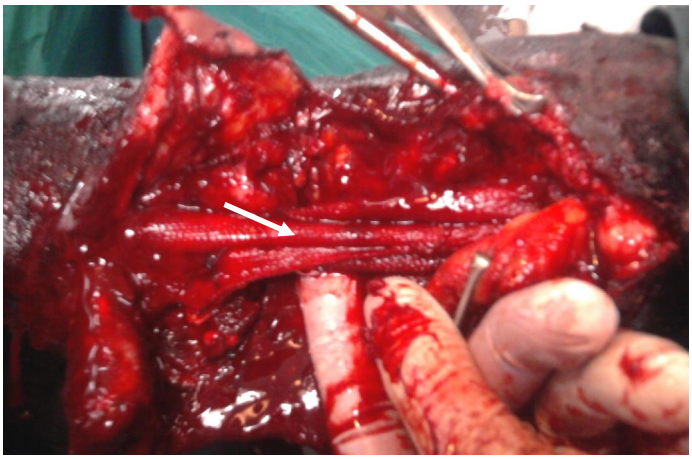


Fig. 15: a case with complete Superficial and Deep digital flexor tendon cuts. Note large gap with massive skin loss.

4. Discussion

Severed and lacerated tendons constitute a high percentage among equine tendon injuries .The condition should be considered serious and even destructive in the field of equine surgery (El – Husseiny;1996 and Stashak;2002).

The use of synthetic prosthetic material was used in different equine replacement surgeries with various results of success (Molloy *et al.*;1991).

The augmentation prosthesis used in this study (polyamide 6.6 \ polyester 1:1) was made to substitute suturing tenorrhaphy in cases of lacerated or cut tendons especially if a gap existed.

Polyamide 100% was previously used for tenorrhaphy and characterized by minimal tissue reaction with low incidence of infection if used in contaminated tissue but its low tensile strength limited its use (Lipowitz;1985). Braided polyester has a high tensile strength compared with polyglycolic acid and polydioxanone (Leknes *et al.*;2005).The new prosthesis was composed of a combination of polyamide and polyester to obtain a biocompatible mesh with a considerable tensile strength. The material was designed in the form of a woven fabric to act as a scaffold for the deposition and orientation of the tendon fibers. It has multiple pores that facilitate the growth of capillary network and consequently oxygen delivery as the collagen synthesis is an oxygen dependent process (Jann *et al.;* 1992). The material was malleable, so it was easily handled while economically, its cost was low.

Clinically, the application of the prosthetic material (polyamide 6.6\polyster)on the experimental and clinical cases revealed no signs of rejection or infection that proved its biocompatibility due to the inert nature of the material and its resistance to bacterial contamination, similar interpretation was mentioned by (Campbell ;1992).

The progression of healing could be seen by ultrasonographic follow up which began by the resolution of the initial peritendineous inflammatory edema and ended by attaining the normal echogenic appearance (Bertone *et al*., 1990). It was noticed that the acoustic shadow of the implant continuously decreased due to the disintegration of the implant with the appearance of the hypoechoic structures which may be attributed to the early fibrosis and granulation tissue formation.

The histopathological examination explained the ultrasonographic findings as the anechoic areas appeared at the seat of implantation were proved microscopically by the presence of newly formed blood capillaries and edema due to the early inflammation. The continuously increasing hypoechoic structures were proved microscopically by granulation tissue formation.

Complete union of the two cut ends of the tendons was clearly noticed at 90 days postoperatively with presence of very small acoustic shadow representing the remnants of the implant which appeared histologically as vacuoles surrounded by macrophage.

It had been postulated that, fragmentation of the implant caused transfer of the tensile loads and cause areas of stress that stimulate collagen synthesis thereby creating mature longitudinally oriented new tendon fibers filling the gap. The same was mentioned by (Sharma ; 2005).

The biomechanical evaluation proved the success of the polyamide implant as the tendon regained its normal features regarding ultimate tensile strength and strain. It was noticed that, the augmentation implant is reported to share stress with the graft tissue during the early collagen remodeling period as was reported by (Hope ; 2007).

Where we use the fabric in the warp direction yarn this has contributed to increase and strengthen the function of tendon, polyester for its unique properties of strength and durability (Gupta; 1998).

The clinical application of the new synthetic material favored its use in the clinical field. Out of 5 animals, 4 returned to their intended use of limb function. Two of them had the cuts in the long digital extensor tendons and revealed good healing as the cuts of the extensor tendons usually heal without complications due to the low weight supported by those tendons. Cases with SDFT cuts also showed good healing as the superficial flexor tendons usually give good prognosis as it carries less of the weight of the animal .On the other hand the case of ruptured SDFT and DDFT failed as the DDF tendon cuts always have guarded prognosis due to they carry most of the animal's weight Especially in heavy weight horses and in such cases the postoperative support play an important role in the success of the operation .The case was more complicated by the presence of a large gap (10 cm) and a massive skin loss. In our opinion the failure had no relation to the application of the prosthetic material and we recommend further studies on a wide scale to investigate the efficiency of the polyamide - polyester use in such cases.

In conclusion, It was found that the new prosthetic material (polyamide 6.6 : polyester) appeared to be biocompatible and gave acceptable outcome when implanted for the repair of tendon deficits in equine . The evaluation of any implanted material should always be accomplished through clinical, ultrasonographic, histopathologic and biomechanical investigations in a way that each investigation explain and interpret the other investigations.

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Synthesis, Isomerization, and Antimicrobial Evaluation of Some IndenothienoPyrimidine Derivatives

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Abstract: 9-Hydroindeno[1′,2′:4,5]thieno[2,3-d]pyrimidin-4-ylhydrazine**(2)**was used as a precursor for preparation of some novel 2-substituted [1,2,4] triazolo[1,5,-c]-9H-indeno [1′,2′:4,5]thieno[3,2-e]pyrimidine derivatives **(5,6,8)**and 9H-indeno[1′,2′,:4,5]thieno[3,2-e]tetrazolo [1,5-c] pyrimidine **(10)**. Furthermore, the preparation of N-substituted-9-H-indeno [1′,2′: 4,5]thieno[2,3-d] pyrimidin-4-yl) amine derivatives**(11-14)** were described. Also, thieno[2,3-d] pyrimidin-4-yl-isothiourea derivatives **15** was obtained from reaction of **1** with thiourea. Selected members of the prepared compounds were screened for antimicrobial activity.
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1. Introduction:

Since resistance to antimicrobial drugs is widespread, there is an increasing need for identification of novel structure lead that may be used in designing new, potent and less toxic antimicrobial agents. Many thienopyrimidine derivatives have been found to posses a wide span of medical activities including antimicrobial activity,[1-6] antiviral,[7-10] antihypertensive,[11]antihistaminic,[12] neurotropic,[13] antidepressant, sedative and analgestic activities.[14,15] Previous observations revealed that the [1,2,4] triazolo[4,3-*c*]pyrimidine derivatives can isomerize under different suitable reaction condition to the thermodynamically more stable [1,2,4]triazolo [1,5-c]pyrimidines.[4,5] This isomerization was reported early by Miller and Rose.[16,17] In continuation of our previous work on thienopyrimidines,[2,7,9] we aimed to synthesize[1,2,4]triazolo[4,3-*c*]pyrimidines and [1,2,4] triazolo [1,5-*c*] pyrimidines not only to study their isomerization, but also to obtain new compounds which are expected to possess notable chemical and biological activities.

2. Experimental

Melting points were recorded on an electrothermal IA 9100 digital melting point apparatus. IR spectra (V_{max} in cm^{-1}) were recorded on a Shimadzu FT-IR 8300 spectrophotometer using KBr pellets technique. ^{1}H-NMR and ^{13}C NMR spectra were recorded using Bruker WM-400 spectrophotometer using DMSO-d_6 as the solvent and TMS as the internal reference (chemist shifts in ppm). The mass spectra were run at 70 eV with a finnigan SSQ7000 spectrophotometer (thermo-insturment system incorporation, USA). The above spectra and elemental analyses were measured at the National Research Center.

Synthesis of N-(Phenyl methylene)indeno[1′,2′:4,5] thieno[2,3-d] pyrmidin-4yl] hydrazine (3).

A solution of compound **2** (2.54 g, 0.01 mol) in ethanol (50 mL) was treated with benzaldehyde (1.06 mL, 0.01 mol) and dropwise of pyridine. The reaction mixture was heated for 15 minutes, cooled, filtered, dried and recrystallized from ethanol to give compound **3**. Yield: 45%, M.P. 211-213°C. ^{1}H-NMR δ 4.0 (s, 2H, CH_2), 4.56 (s, 1H, CH aliphatic) 6.9-7.73 (m, 5H, phenyl), 7.12-7.34 (m, 4H, Ar-H+NH, D_2O exchangeable), 8.04 (d, 1H, J=7Hz, Ar-H), 8.65 (s, 1H, C_2-H). Anal. Calcd. For $C_{20}H_{14}N_4S$ (342.42); C, 70.15; H, 4.12; N, 16.36; S, 9.36%. Found, C, 70.29; H, 4.34; N, 16.57; S, 9.66.

Synthesis of 2-Phenyl-9*H*-indeno [1′,2′:4,5] thieno [3,2-e] [1,2,4] triazolo[1,5-c]pyrmidine (5).

A solution of compound **3** (3.42 g, 0.01 mol) was treated with pyridine drop wise in glacial acetic acid (50 ml) were refluxed for 3h. The reaction mixture was cooled, filtered, dried, and recrystallized from dioxane to give compound **5** . Yield: 40%, M.P. 234-236°C. ^{1}H-NMR δ: 4.01 (s, 2H, CH_2), 7.2-7.59 (m, 5H, phenyl-H), 7.69-7.9 (m, 3H, Ar–H), 8.03 (d, 1H, J= 7Hz, Ar–H), 8.13 (s, 1H, C_5–H), MS.: m/z%=340 (M^+, 5), 82 (100), 156 (16.55) 155 (91.52),

152 (2.43), 87 (2.05), 83(9.16) 70(28.31), Anal. Calcd. For $C_{20}H_{12}N_4S$ (340.40); C, 70.57; H, 3.55; N, 16.65; S, 9.53% Found, C, 70.58; H, 3.56; N, 16.67; S, 9.55%.

Synthesis of 2-Amino-9*H*-indeno[1′,2′:4,5]thieno [3,2-e] [1,2,4] triazolo[1,5-c]pyrmidine (6).

Compound **2** (2.54 g, 0.01 mol) was dissolved in (50 mL) glacial acetic acid, then potassium thiocyanate (0.97g , 0.01 mol) was added. The reaction mixture was refluxed for 6h, cooled, filtered, washed with water then ethanol, dried and recrystallized from methanol to give compound 6. Yield: 61%, M.P. 231-233°C IR υ: 3329.78, 3229cm^{-1} (NH_2) ^{1}H-NMR δ: 4 (s, 2H, CH_2), 7.2-7.59 (m, 3H, Ar-H), 8.1 (d, 1H, J=7Hz, Ar-H), 8.77 (s, 1H, C_5-H), 9.8 (br, 2H, NH_2), 14.1 (br, H, NH, D_2O exchangeable) MS: m/z% 280(M^+, 35), 256 (19.61); 240 (25.13), 196(41.56), 44(100). Anal. Calcd for $C_{14}H_9N_5S$ (280.32); C, 60.20; H, 3.25; N, 25.07; S, 11.48%. Found, C, 60.21; H, 3.26; N, 25.09; S, 11.50.

Synthesis of 2-N-[thien-2-yl) methyleneamino-9*H*-indeno[1′,2′:4,5] thieno [3,2-e] [1,2,4] triazolo[1,5-c]pyrmidine (7).

A solution of compound **6** (2.81g, 0.01 mol) and 2-thiophene aldhyde (1.12g., 0.01 mol) and drop wise of pipyridine in ethanol (50 ml) was refluxed for 3h. The reaction mixture was cooled, filtered off, dried, and recrystallized from dioxane to give compound **7**. Yield 56%, MP: 212-214°C. IR υ: 1605.4 (C=N) cm^{-1} , ^{1}H-NMR δ: 4.08 (s, 2H, CH_2), 5.2 (s, 1H, aliphatic CH), 7.04-7.3 (m, 3H, thiophene), 7.29-7.31 (m, 3H, Ar-H), 8 (d, 1H, J= 7Hz, Ar-H), 8.19 (s, 1H, C_5-H), MS: m/z% 373 M^+ 6.35), 334(4.04), 313 (1.88), 256(100). Anal. Calcd for $C_{19}H_{11}N_5S_2$ (373.45); C, 61.11; H, 2.97; N, 18.75; S, 17.17%. Found C, 61.32; H, 3.12; N, 18.76; S, 17.31.

Synthesis of 3-Methyl-9H-indeno [1′,2′: 4,5]thieno [3,2-e][1,2,4]triazolo [1,5-c] pyrimidine (8).

Compound **2** (2.54g, 0.01 mol) was heated under reflux in 50 ml acetic acid for 6 h; then the reaction mixture was cooled, filtered, dried and recrstallized from acetone to give compound **8**. yield 57%, M.P. 243-245°C. IR, υ 1612.2 cm^{-1}(C=N). ^{1}H–NMR δ: 2.62 (s, 3H, CH_3), 4.12 (s, 2H, CH_2), 7.22-7.43 (m, 3H, Ar–H), 8.2 (d, 1H, J=7Hz, Ar–H), 8.6 (s, 1H, C_5–H). MS: m/z%= 278 (M^+, 18.38), 289(41.9), 224 (4.11), 198(1.57). Anal. Calcd for $C_{15}H_{10}N_4S$(278.33); C, 64.73; H, 3.62, N; 20.13; S, 11.52%, Found C, 64.84; H, 3.82; N, 20.34; S, 11.73.

Synthesis of 3-Methyl-9*H*-indeno [1′,2′:4,5]thieno [3,2-e][1,2,4]triazolo [4,3-c]pyrimidine (9).

A solution of compound **2** (2.54g, 0.01 mol) in 20 mL triethyl orthoacetate was heated under reflux temperature for 10 h. The reaction mixture was cooled, filtered, dried and the product was recrystllized from ethanol to give compound **9**.

Isomerization of compound 9 to 8

A solution of compound **9** (2.78g, 0.01 mol) in ethanol (30mL) in the presence of a few drops of piperidine was heated under reflux temperature for 30 min. the solvent removed under reduced pressure leaving a solid product which was recrystallized from ethanol to give a compound identical in all aspects with compound **8**.

Synthesis of 9*H*-Indeno[1′,2′:4,5]thieno[3,2-e] tetrazolo [1,5-c]pyrmidine (10).

A cooled solution of compound **2** (2.54 g, 0.01 mol) in diluted HCl (20 mL) was treated drop wise with a cooled solution of sodium nitrite (prepared from 1g sodium nitrite dissolved in 15 mL water), then stirred at room temperature for 2h. The separated solid was filtered off, dried, and recrystallized from dioxane to give **10**. Yield : 58%, M.P. 211-213°C: IR υ: 1612.2 cm^{-1} (C=N). ^{1}H-NMR δ: 3.97 (s, 2H, CH_2), 7.12-7.6 (m, 3H, Ar–H), 8.4 (d, 1H, J=7 Hz, Ar-H), 9.4 (s, 1H, C_5-H) MS: m/z% 265(M^+, 6.09), 237 (16.54), 236 (17.94), 63 (100). Anal. Calcd. For $C_{13}H_7N_5S$ (265.29); C, 58.86; H, 2.66; N, 26.4; S, 12.09%. Found : C, 58.87; H, 2.67; N, 26.5; S, 12.11.

Synthesis of 4-N-Substituted (indeno[1′,2′:4,5] thieno[2,3-d] pyrimidine 11-14: General procedure

To a solution of compound **1** (2.58g., 0.01 mol) in ethanol (30 mL), aniline, phenyl hydrazine, aminoacetic acid or ethanol amine (0.01 mol) was added respectively. The reaction mixture was heated for 2-6h. then the formed precipitate was filtered off and recrystallized from ethanol to give compounds **11-14**.

Phenyl (9H-Indeno[1′,2′:4,5]thieno[2,3-d] pyrimidin- 4-yl) amine (11).

Yield after 2h: 81%, M.P. 209-211°C. IR υ: 3438 (NH) cm^{-1}. ^{1}H-NMR δ: 4.1 (s, 2H, CH_2), 7.06-7.17 (m, 5H, phenyl), 7.25-7.83 (m, 3H, Ar–H), 7.85 (d, 1H, J=7Hz, Ar–H), 8.56 (s, 1H, C_2-H) 9.41 (br, 1H, NH, D_2O exchangeable) MS: m/z% 315 (M^+, 12.53), 258 (100), 224 (15.43). Anal. Calcd for $C_{19}H_{13}N_3S$ (315.39); C, 72.36; H, 4.15; N, 13.32; S, 10.17%. Found: C, 72.38; H, 4.17; N, 13.33; S, 10.19.

N-Phenyl-N\'-(9H-indeno[1′,2′:4,5]thieno[2,3-d] pyrimidin-4-yl)hydrazine (12).

Yield after 2h: 83%, M.P. 205-207°C. ^{1}H NMR δ: 3.64 (s, 2H, CH_2), 6.82-6.94 (m, 5H, phenyl), 7.20-7.26 (m, 3H, Ar–H), 7.8 (d, 1H, J = 7Hz, Ar–H), 7.9 (s, 1H, C_2–H), 8.2 (br, 1H-NHNHPh) , 9.42 (br, 1H, NHNHPh), and NH, D_2O exchangeable. Anal Calcd for $C_{19}H_{14}N_4S$ (330.41); C, 69.07; H, 4.27; N, 16.96; S, 9.70. Found, C, 69.10; H, 4.29; N, 16.98, S, 9.72.

N-(9H-Indeno[1′,2′:4,5]thieno[2,3-d]pyrimidin-4-yl) amino acetic acid(13).

Yield after 6h: 51%, M.P. 214-216°C. IR: υ 1713.12 cm^{-1} (C=O), 3443.18 broad (NH, OH). MS: m/z 297.33, (M^+, 20%) 280 (6.7), 225(1.44), 142 (12.100). Anal calad. For $C_{15}H_{11}N_3O_2S$ (297.33); C, 60.59; H, 3.73; N, 14.13 ; S, 10.78%. Found, C 60.60; H, 3.74; N, 14.14; S, 10.80.

2-(9H-Indeno[1′,2′:4,5]thieno[2,3,-d]pyrimidin-4-yl amino)-4-ethanol (14)

Yield after 3h: 86%, M.P. 207-209°C. ^{1}H-NMR δ: 3.66 (t, 2H, J = 8.0, $CH_2\underline{CH_2}OH$), 3.73 (t, 2H, J = 8.0, $CH_2\underline{CH_2}OH$), 4.0 (s, 2H, CH_2), 5.0 (s, 1H, OH, D_2O exchangeable), 6.78 (br, 1H, NH, D_2O exchangeable), 7.26-7.85 (m, 3H, Ar-H), 7.93 (s, 1H, J=7 Hz, Ar–H), 8.39 (s, 1H, C_2H).^{13}C NMR δ: 37(1C, indeno C), 44.06 (1C, aliphatic NH$\underline{C}$), 60.27 (1C, aliphatic $\underline{C}H_2O$) 115-125.7 (6C, phenyl), 121-127 (6C, Ar–C), 141.8-146.99 (4C, thiophene), 153.3 (1C, C_2 pyrimidine), 157.97 (1C, pyrimidine $\underline{C}$NHNH) Anal. Calcd. For $C_{15}H_{13}N_3OS$ (283.25); C, 63.58; H, 4.62; N, 14.83; S, 11.32%. Found, C, 63.59; H, 4.64; N, 14.84; S, 11.34.

Synthesis of 2-(9H-Indeno[1′,2′:4,5]thieno[2,3-d] pyrimidin-4-yl) isothiourea (15).

A mixture of compound **1** (2.58g, 0.01 mol), thiourea (0.76g, 0.01), sodium hydroxide (0.4g, 0.01 mol) in 30 ml ethanol was stirred at room temperature for 4h. The reaction mixture was filtered dried and recrystallized from methanol to give compound **15**. yield 82%, M.P. 204-206°C. ^{1}H NMR δ: 4.13 (s, 2H, CH_2), 7.29 (br, 2H, NH_2, S_2O exchangeable), 7.34-7.83 (m, 3H, Ar-H), 8.1 (s, 1H, J = 7 Hz, Ar-H), 8.8 (s, 1H, C_2-H) 10.8 (br, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{14}H_{10}N_4S_2$ (298.39); C, 56.35; H, 3.38; N, 18.78; S, 21.49%. Found C, 56.36; H, H 3.39; N, 18.79; S, 21.51.

Agar Diffusion Medium:

A suspension of the organisms were added to sterile nutrient agar media at 45 °C and the mixture was transferred to sterile petri dishes and allowed to solidify. Holes of 10 mm in diameter were made using a cork borer.) An amount of 0.l ml of the synthesized compounds was poured inside the holes. A hole filled with DMSO was also used as control. The plates were left for 1 hour at room temperature as a period of pre-incubation diffusion to minimize the effects to variation in time between the applications of the different solutions. The plates were then incubated at 37°C for 24 hours and observed for antibacterial activity. The diameters of zone of inhibition were measured and compared with that of the standard, the values were tabulated. Ciprofloxacin (50 µg/ml) and Ketoconazole (50 µg/ml) were used as standard for antibacterial and antifungal activity respectively. The observed zone of inhibition is presented in Table (1).

Minimum Inhibitory Concentration:

Minimum Inhibitory Concentration (MIC) of the tested compounds were determined by agar streak dilution method. 68mg/ml stock solution of the synthesized compounds were made using DMSO as the solvent. From this stock solution, the following concentrations (0.17; 0.34; 0.68; 0.85 and 1.7 mg/ml) of the solutions of the tested compounds were mixed with the known quantities of molten sterile agar media aseptically. About 20 ml of the media containing the tested compound was dispensed into each sterile Petri dish. Then the media were allowed to get solidified. Microorganisms were then streaked one by one on the agar plates aseptically. After streaking alphe-plates were incubated at) 37°C for 24 h/48 h. for bacterial and fungus activity respectively. Then the plates were observed for the growth of microorganisms. The lowest concentration of the synthesized compounds inhibiting the growth of the given bacteria/fungus was considered as minimum inhibitory concentration (MIC) of the test compounds against that bacteria or fungi on the plate is presented in Table (2).

3. Results and Discussion

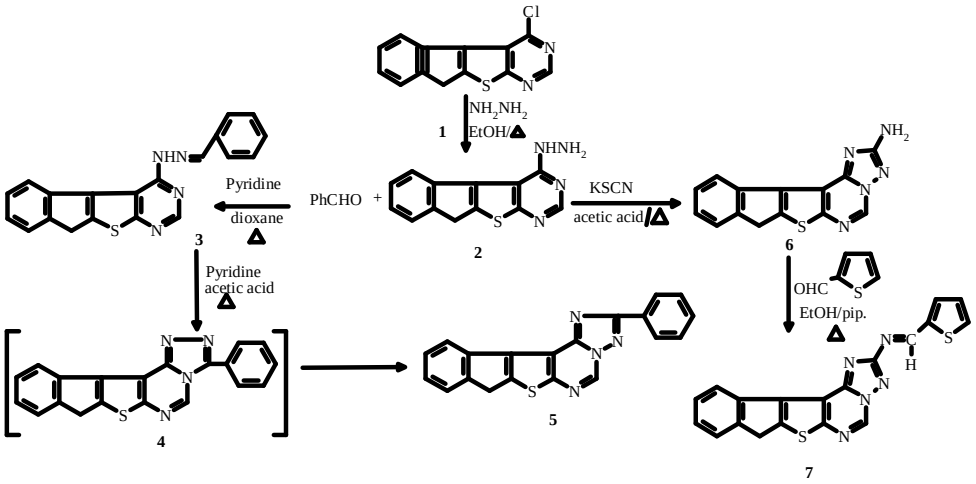
Chemistry

Indeno[1',2':4,5]thieno[2,3-d]pyrimidin-4-yl hydrazine **(2)**, as key compound for this study and for further syntheses of other fused heterocyclic compounds, was previously synthesized by Hegab *et al.*[(5)]. Through the reaction of 4-chloroindeno [1',2':4,5] thieno[2,3-d]pyrmidine **(1)** with hydrazine hydrate in ethanol under reflux temperature. Condensation of hydrazino derivative **2** with benzaldehyde in the presence of few drops of pyridine took place by heating under reflux in ethanol where the corresponding hydrazone **3** was produced. Attempts to cyclize the latter compound to its corresponding 2-phenyl [1,2,4] triazolo[1, 5-c] pyrimidine derivative **5** by refluxing with pyridine in glacial acetic acid took place presumably via the isomerization of intermediate [1,2,4] triazolo[4,3-*c*]

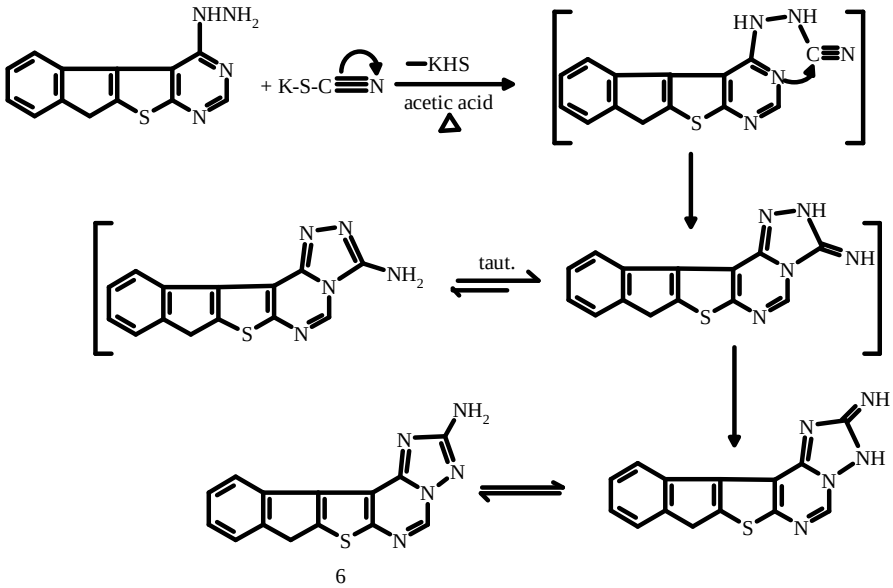
pyrimidine **4** according to dimroth-type rearrangement.[18] The structures of compounds **3** & **5** (Scheme 1) were confirmed on the basis of their elemental and spectral data. The ^{1}H NMR spectrum of product **5** showed the absence of the signals for N=CHPh and NH protons which appeared in the spectrum **3** at δ 4.56 & 7.12 ppm, respectively. Moreover, the C_5-H proton of product **5** which appeared at δ 8.13 favors its structure over structure **4** since the C_5-H proton of **4** is more deshilded and would have appeared at a higher value.

4-Hydrazino derivative **2** reacted with potassium thiocyanate in boiling acetic acid to give 2-aminoindeno[1',2':4,5]thieno[3,2-e] triazolo[1,5-c] pyrimidine **(6)** (scheme 1). The reaction may be accomplished via the intermediates as shown in the considered mechanism (Scheme 2).

Meanwhile, condensation of 2-amino derivatives **(6)** with thiophene-2-aldhyde in the presence of few drops of piperidine in ethanol gave the corresponding schiff's base **(7)** (Scheme 1), the spectral data of compounds **6** & **7** are in agreement with assigned structures. Their IR spectra showed the absence of the presences of absorption band for NH_2 group at 3329 & 3229cm^{-1} and presence of aliphatic CH signal at δ 5.2 ppm in their ^{1}H-NMR spectrum of compound **7**.



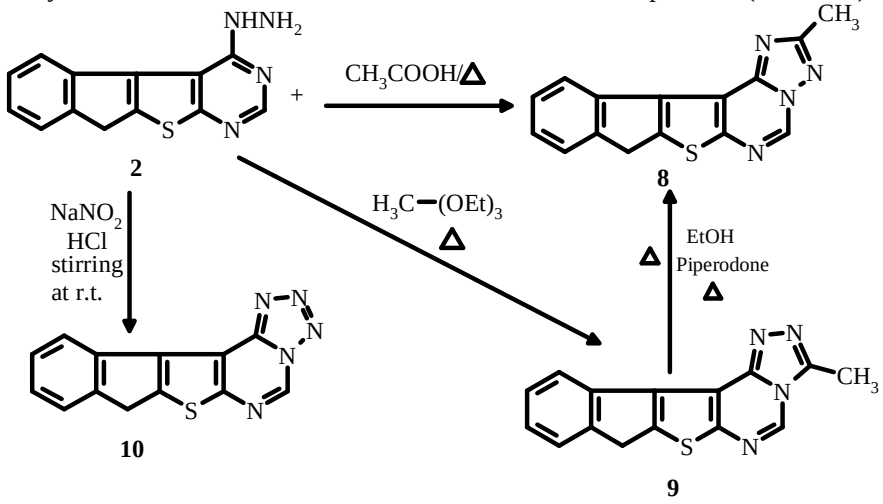
Scheme (1)



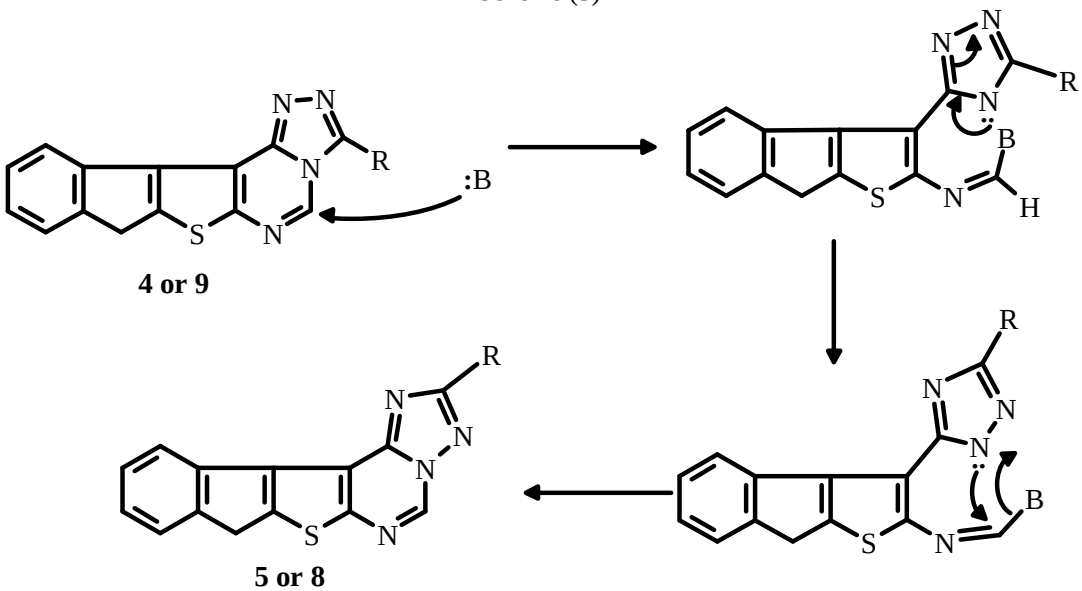
Scheme (2)

Many reports[4,19] have described the synthesis of [l,2,4]triazolo[4,3-c]pyrimidine derivatives and [l,2,4]triazolo[l,5-c]pyrimidine fused to different nitrogen containing heterocyclic moieties. In the meantime, the synthesis of [l,2,4]triazolo[4,3-c]pyrimidine derivatives and [l,2,4] triazolo[l,5-c]pyrimidine derivatives fused to a thiophene moiety have attracted the attention of many investigators.[20-23] Actually, previous observations revealed that thieno[2,3-e] [l,2,4] triazolo [4,3-c]pyrimidines can isomerize under different suitable reaction conditions to the more stable thieno[3,2-e][l,2,4]triazolo[l,5-clpyrimidines[4,5]. Nevertheless, this pattern of isomerization appears to have been overlooked by some workers[21,24].

In this investigation, when compound **2** was heated under reflux temperature in acetic acid, it afforded[1,2,4]triazolo[1,5-c]pyrimidine derivative **(8)** probably via the intermediacy of its isomer[1,2,4]triazolo[4,3-*c*] pyrimidine (**9)** which was not isolated in this reaction, but underwent a Dimroth-type rearrangement[18] under the conditions of the reaction. Compound **9** was reported previously by Hegab *et al.*[4] through the reaction of compound **2** with triethyl orthoacetate under reflux temperature, it afforded [1,2,4]triazolo[4,3-*c*]pyrimidine **(9)** which was converted into the more stable its isomer **(8)** by heating in ethanolic piperidine which presmably involves a sequence of ring opening and ring closure reactions as depicted in (Scheme 4).



Scheme (3)

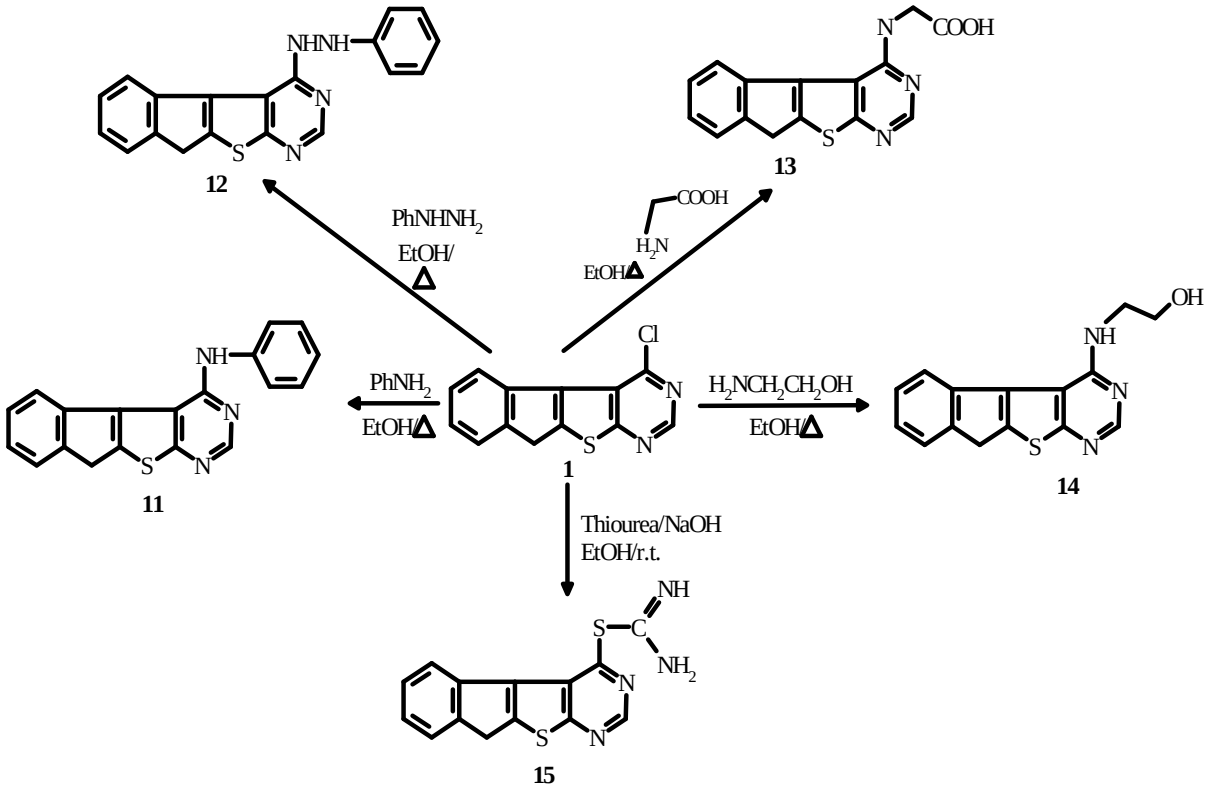


Scheme (4). Rearrangement of compounds **4** and **9** to **5** or **8**

Heterocyclic azides, especially azidomethines, can exist in equilibrium with their tetrazolo tautomers and this equilibrium affected by many factors: pH, temperature, the nature of the substituents around the (C=N) and the used solvent.[25,26] This equilibrium can be shifted in either direction by controlling these factors, and the IR spectroscopy is helpful in revealing which form is predominant, since the azido structure can show a characteristic band in the region υ2220-2320cm^{-1}. Thus, nitrosation of the hydrazino group of compound **2** afforded indeno[1',2':4,5] thieno [3,2-e] tetrazolo [1,5-*c*] pyrimidine **(10).** Analytical and spectral data are in agreement with the proposed structure, IR spectrum did not show absorption frequency indicative for the azido group (cf. Exp.).

Also, the reactivity of chloropyrimidine **1**[5] towards N-nucleophiles, namely, aniline, phenyl hydrazine, ethanol amine in absolute ethanol was investigated which yielded the respective substituted amine derivatives **11-14**. (Scheme 5). The structures of the latter compounds were confirmed on the basis of their elemental analysis and spectral data. The IR spectra of the compounds showed absorption bands characteristic for NH group and ^{1}H-NMR spectrum of compound **14** as an example, revealed signals at δ: 3.66 (t, 2H, J = 8.0 Hz, $\underline{CH_2}CH_2OH$), 3.73 (t, 2H, J = 8.0 Hz, $CH_2\underline{CH_2}OH$), 4.0 (s, 2H, CH_2), 5.0 (s, 1H, OH, D_2O exchangeable), 6.78 (br, 1H, NH, D_2O exchangeable), 7.26-7.85 (m, 3H, Ar-H), 7.93 (d, 1H, J = 7 Hz, Ar-H), 8.39 (s, 1H, C_2-H) ^{13}C NMR δ: 37 (1C, indeno CH_2), 44.06 (1C, aliphatic NHC), 60.27 (1C, aliphatic CH_2O), 115-125.7 (6C, phenyl), 121-127 (6C, Ar-C), 141.8-146.99 (4C, thiophene), 153.3 (1C, C_2 pyrimidine), 157.97 (1C, pyrimidine $\underline{C}$NHNH).

On the other hand, when a mixture of compound **1**, thiourea and sodium hydroxide in ethanol was stirred at room temperature[5], it gave isothiourean-4-yl indeno [1',2' : 4,5] thieno[2,3-d]pyrimidine **(15)** (scheme 5)



Scheme (5)

Antimicrobial activity:

The antibacterial activity of the synthesized compounds was tested against *Escherichia coli* NRRL B-210 (Gram -ve bacteria), *Bacillus subtilis* NRRL B-543 (Gram +ve bacteria), *Staphylococcus aureus* NRRL B-313 using nutrient agar medium. The antifiingal activity of the compounds was tested against *Candida albicans* NRRL Y-477 using Sabouraud dextrose agar medium. The results are summarized in Table 1 & 2.

Table (1): Inhibition zone in mm as a criterion of antibacterial and antifungal activities of the newly synthesized compounds

Compound No.	Minimum inhibitory concentration in mg/ml			
	Gram positive bacterial		Gram negative bacteria	Fungi
	Bacllus subtilis	*Staphyococcus aureus*	*Escherichia coli*	*Candida albicans*
2	25	22	22	22
3	23	22	21	23
5	18	21	22	22
6	13	-ve	-ve	-ve
7	17	23	13	-ve
8	23	21	23	25
10	22	23	16	25
11	13	17	-ve	12
12	17	18	-ve	17
13	25	25	25	25
14	12	20	21	-ve
15	25	25	25	25
Reference drugs				
Ciprofloxaxine (µg/ml)	23	23	25	-ve
Ketaconazole(µg/ml)	-ve	-ve	-ve	23

Table (2): MIC in mg/ml of the newly synthesized compounds

Compound No.	Minimum inhibitory concentration in mg/ml			
	Gram positive bacterial		Gram negative bacteria	Fungi
	Bacllus subtilis	*Staphyococcus aureus*	*Escherichia coli*	*Candida albicans*
2	1.25	0.625	0.625	
3	0.625	0.625	0.625	
5	0.625	0.625	0.625	
6	0.625	0.625	0.625	
7	1.25	1.25	1.25	
12	1.25	1.25	1.25	
14	1.25	1.25	1.25	
Reference drugs				
Ciprofloxaxine (µg/ml)	0.12	0.15	0.01	--
Ketaconazole(µg/ml)	--	--	--	0.03

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Economic Analysis of Foreign Trade between Egypt and countries of the Great Arab Free Trade Agreement

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Abstract: Establishment of Great Arab Free Trade Agreement (GAFTA) is considered one of the most important economic achievements among Arab countries, where the declaration of GAFTA is an important factor to accelerate the process of canceling of tariff and non-tariff barriers on trade among Arab countries. The research problem is concerned with the volume of trade between Egypt and GAFTA countries is relatively small, so the object of this research is to try to access how to increase the volume of trade between Egypt and country members in the GAFTA, and to identify the most important variables affecting Egyptian exports and imports with GAFTA, and to identify the most important countries that are expanding or limiting in imports from Egypt. The results showed that Saudi Arab was ranked as the first in the list of Egyptian exports, which ranged between 21.7%, 20.4% during 2005, 2009. As it turns out that Kuwait was as the ranked first in the list of Egyptian imports in 2005 by 33.6% of the total value of Egyptian imports from these countries. Exports of Saudi Arab to Egypt were in the first rank during the period (2006-2009) by 37.8%, 48.4%, 44.3%, 42.3%, respectively. The results of the gravity model showed that an increase in per capita GDP in each of Egypt and GAFTA countries lead to increase exports and imports, while increasing the geographical distance between Egypt and GAFTA countries lead to a decrease both of them. Gravity model for exports showed that to Sudan and Syria response to the demand for Egyptian exports at high levels of income of individuals, while the gravity model for imports showed the Egypt response to import from Saudi Arabia, UAE, Libya, and Kuwait. The study therefore recommended the need to develop joint infrastructure projects, and improve the means of transport, particularly two states, with neighboring Libya and Sudan, and export of good and services take into account consumer taste and quality requirements.

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Key Words: Great Arab Free Trade Agreement (GAFTA) Gravity Model Egyptian Exports, Egyptian Imports, open door policy.

Introduction:

The importance of Great Arab free trade Agreement is that it will prove a sort of balance among the Arab economic integration through the permeability between the Arab markets, and the open door policy on the world economy, and also the different international trading Blocks due to the integrally of Arab markets will lead to establish some projects for the development of industrial, agricultural and other sectors to face the challenges of world trade association and the flowing of investments between the member countries, and benefiting from the huge Arab markets and establishing of mutual Arab investments, that achieve Arab development aiming to achieve the common Arab market, the establishment of the Great Arab free trade Agreement is considered one of important economic achievements on the common Arab work level, where the Arab countries began to liberate all the Arab commodities that are exchanged between them gradually since 1998, through decreasing custom, duties and taxes, which have the similar impact by 10% per year reaching to the full liberalization of all exchanged Arab commodities in the year 2005. The declaration of the Great Arab free trade Agreement forms an important factor accelerate the process of releasing custom duties and other duties in the exchange trade and improving exchange terms between Arab countries.

Research Problem:

The research problem is that the volume of the exchanged trade between Egypt and members of the Great Arab free trade Agreement is relatively small which reflect its impact in the difficulty for getting hard currency which is needed to push economic development cycle where the Egyptian exports and imports value imports with the Great Arab free trade Agreement members were estimated 8.2 and 6.3 milliard dollar represents 31.8% and 12.2% from the total Egyptian exports and imports that are about 25.8 and 51.8 milliard dollar in 2009.

Research Aim:

Due to Egypt has a positive trade balance with the Great Arab free trade Agreement estimated members about 1.9 Milliard dollar in the year 2009, so the research aims to the possibility of raising the exchange value between Egypt and the Arab countries, through acknowledge of the most

important countries that can expand or limit its imports from Egypt, with the countries of the Great Arab trade Agreement and present some suggestions which help to increase commercial exchange between Egypt and (GAFTA) members. .

Research Method and Data Sources:

The "Gravity Model" was estimated by using the (section data) and "Panel Data" regression method also the data had been collected from the central authority for general mobilization statistics and also the web site of the "International Bank" during the period (1995-2009).

Advantages of the Great Arab free trade Agreement:

1. Protection of the Arab Economic interests, also activating the trade between them in a form working to raise the level of the Arab Economy especially that the Great Arab trade Agreement is considered an important take-off facing a gradual liberalization which lead to the liberation of securing the transportation of the production elements between the Arab countries and the distinguish usage of it, an activating in a useful way working to transfer it to an Arab custom union reaching to an Arab common market.
2. The Importance of this region is concealed in uniting and concluding the Arab markets through comprehending the new technology and directing towards the production, reducing the costs, creating new work opportunities, distinguishing distribution of resources, better integration of the industries, and the open door policy on the world Economy with confidence.
3. Facilitating the gradual integration process among the new trade systems and creating a unique Arab distinction to deal with the requirements of the world trade Organization and the different international trade masses, especially that the unifying of the Arab markets will lead to establishing some projects for the agricultural and industrial development to face the challenges of the agreements of the world trade organization, to support the confidence between the local and the foreign investors and also to inflow the investments among the member countries, the attraction of the direct investments from a broad and polarization of the projects of technological transfer, will contribute to the consolidation of Arab economic development which lead to realizing the hopes related to the Arab Common Market.
4. To facilitate the trade between the Arab countries members in the Great Arab free trade Agreement. Where the approval was cancelled on the certificates of source (origin) and all the attached certificates from the embassies and consulates of the countries members as the distinguished treatment, also, the countries which is conform canceling legalization for the certificate of origin are : Saudi Arabia, Egypt, Sudan, sultanate of Oman, Bahrain, Kuwait, Lebanon, Morocco, Tunis, Syria. The United Arab Emirates).
5. The gradual reducing in the custom duties and the taxes which have similar affection, where it was declared the vary to liberate all the Arab commodities that are exchanged among the member countries which started from January 1998, through the reducing the custom duties, and the other taxes with have the same affection and hazing in each member country with the Beginning of applying the agreement by a percentage of 10% per year, and in a form led to achieve the complete liberation for all the Arab commodities with the beginning of the year 2005. Libya has cancelled all this fees since the beginning of the agreement; also Palestine was from the applying of the gradual reducing (The Arab summit- non usual- agreement No. 355 in the data 22-10-2000).

   As for Sudan, started reducing the fees on the exports with the Sudanese origin by 60% since January 2003, reading to 100% percentage since January 2005. As for the Arab origin imports in Sudan, it have been collected all custom duties completely since the end of the year 2004 there for began the gradual reducing by 20% percentage from January 2005 reaching the complete liberation in January 2010 (The Arab Summit –No. 233 in 28-3-2002) The gradual began in Yemen from January 2005 with a percentage of 16% yearly until January 2009, then it became 20% in the beginning of 2010, and to be totally admitted at the end of that year.

The obstacles of processing the agreement of the Great Arab Free Trade Agreement:

1. The non-custom restricts either technical or administrative, the most important of it is the overestimation of custom re-evaluation fees, the complications in the procedures of crossing the borders, over-estimating the period spent in checking the samples and the inspection procedures, in addition, the over-estimation of the requirements of specifications and measurements, the strict limitation on weight control, the complications in the procedures of detecting and inspection and the medical procedures.
2. The fees and adwinshative and technical dues similar to the custom duties that prevent achieving the expected hopes from the area (region), especially it shows a progressive increase than the previous time especially in the sphere the overland freight and the transit.

3. The not reading of a decision for detailed rules of origin, unified for all the Arab origin commodities.
4. The continuation of some member countries of the exception of the gradual reducing the custom fees, where Egypt reject it cancellation with the condition of putting the unique detailed rules of origin for the Arab commodities.
5. The non-commitment of some member countries with the adjudicates of the executive program, like Sudan which didn't commit diving the gradual reducing of custom fees and taxes of the same impact by a percentage of 16% beginning of the year 2005, it has been late because the renew and the development on the Sudanese yard related to the definition of the peace agreement in Sudan which has opposed new burdens on the Sudanese government budget.
6. The non-completion of all the Arab countries to Join the agreement, Algeria and Mauritania didn't complete the procedures of joining the (GAFTA) and, there three are Arab countries didn't join the (GAFTA) yet, these are Djibouti, Somalia and the Moon Islands.
7. The insistence of some member countries to the conditioning of the approval on the certificates of origin from its embassies and consulate like Qatar united Emirates and Libya, although it was a plural decision of canceling this condition.
8. The imposed restrictions on the currency exchange in some Arab countries through paying exaggerated dues like Egypt, Syria and Iraq.
9. The decrease of the freight costs in some Arab countries and the connections between the member countries.
10. The difficulty of gaining visas to some Arab countries like Saudi Arabia, Kuwait, Morocco and the United Emirates.

The Recent situation of the foreign trade between Egypt and the Arab countries:

The Egyptian exports are likely to be of a relative variation comparing with the imports as follows:

The development of the Egyptian exportation value to the most important country members in the Agreement (GAFTA):

The Table (1) shows the evolution of the Egyptian exports in Million dollars to the most important Arab countries during the period (2005-2009). Where it was occurred that the Saudi Arabia had occupied the first standing in the Egyptian exports list that was from about 21.7% in the year 2005 to about 20.4% in the year 2009 of the total value of Egypt's exports to these countries.

In the second standing, is the United Emirates with about 17.4%, 16.1% in the years 2005-2006 squinty, then Libya occupied the second standing in the years 200-2009 by about 14.6%, 15.1% in sequence.

Algeria, had occupied the last standing on the Egyptian export list in the years 2005, 2006 by a percentage of 1.8% and 2.0% squinty, while Kuwait had occupied the last standing in the years 2007, 2008 and 2009 by a percentage of 2.5%, 3.1% and 2.6% in sequence.

Table (1): The value of the Egyptian exports to the member countries in Great Arab Free Trade Agreement (GAFTA).

Exp	2005	%	2006	%	2007	%	2008	%	2009	%
Algeria	31.3	1.8	36.8	2.0	59.3	2.8	189.8	3.4	364.3	5.6
United Emirates	305.8	17.4	302.7	161.1	198.3	9.4	530.0	9.6	558.6	8.5
Jordan	196.4	11.2	249.3	13.3	301.8	14.3	717.0	13.0	933.9	14.3
Kuwait	39.0	2.2	64.7	3.4	53.0	2.5	168.8	3.1	169.9	2.6
Lebanon	174.0	9.9	204.0	10.9	326.7	15.5	419.3	7.9	441.1	6.8
Libya	149.8	8.5	168.6	9.0	247.8	11.7	803.1	14.6	987.8	15.1
Morocco	85.9	4.9	84.8	4.5	169.7	8.0	339.7	6.2	359.0	5.5
Saudi Arabia	381.8	21.7	318.4	16.9	384.0	18.2	1247.4	22.6	1330.0	20.4
Sudan	185.2	10.5	194.6	10.4	165.3	7.8	545.8	9.9	563.4	8.6
Syria	207.4	11.8	255.7	13.6	208.0	9.8	557.7	10.1	828.1	12.7
Total	1756.7	100	1879.5	100	2113.7	100	5521.5	100	6533.0	100

Source: central authority for general mobilization & statistics, between of foreign trade- separate editions.

Table (2): The value of the Egyptian imports to the member countries in Great Arab Free Trade Agreement (GAFTA)

Exp	2005	%	2006	%	2007	%	2008	%	2009	%
Algeria	694.5	27.6	601.7	16.0	369.8	8.0	495.2		364.3	5.6
United Emirates	137.2	5.5	131.4	3.5	191.0	4.1	881.9		558.6	8.5
Jordan	72.6	2.9	85.3	2.3	66.0	1.4	108.1		933.9	14.3
Kuwait	843.0	33.6	1084.0	28.8	1244.6	26.9	1603.8		169.9	2.6
Lebanon	66.0	2.6	73.7	2.0	102.0	2.2	171.0		441.1	6.8
Libya	119.2	4.7	102.9	2.7	197.9	4.3	261.5		987.8	15.1
Morocco	11.0	0.7	14.0	0.4	23.3	0.5	38.4		359.0	5.5
Saudi Arabia	357.7	14.2	1425.3	37.8	2243.5	48.4	3115.1		1330.0	20.4
Sudan	64.6	2.6	60.2	1.6	50.5	1.1	48.9		563.4	8.6
Syria	146.5	5.8	190.2	5.0	146.2	3.2	306.0		828.1	12.7
Total	2512.2	100	3768.6	100	4634.8	100	7029.7		6533.0	100

Source: central authority for general mobilization & statistics, between of foreign trade- separate editions.

The development of the Egyptian importation value from the most important country members in the (GAFTA).

Table (2) shows the evolution of the value of the Egyptian imports in Million dollars from the most important Arab countries during the period (2005-2009), where it occurred that Kuwait had occupied the first standing in the Egyptian imports list in the year 2005 by a percentage of 33.6% of the total imports of Egypt from these countries.

Also exports of Saudi Arabia to Egypt had come in the first standing during the period (2006-2009) by a percentage of 37.8%, 48.4%, 44.3% and 42.3% squinty.

The Kuwait's exports to Egypt the second standing during the period (2006-2009) by a percentage of 28.8%, 26.9% 22.8% and 24.2% in sequence.

Morocco had occupied the last standing in the Egyptian imports list during the period (2005-2008) with a percentage of 0.4%, 0.4%, 0.5% and 0.5% in sequent while Sudan occupied the last standing in the year 2009 with a percentage of 0.9%.

Measuring the economic trade exchange between Egypt and the Great Arab free trade Agreement .

By estimating the gravity Model for trade exchange between Egypt and the most important countries of the great Arab free trade Agreement in both cases of exports and imports they are (Algeria- United Emirates – Kuwait-Lebanon-Libya Saudi Arabia- Sudan- Syria) during the period (1995-2009), where Egypt export to them about 80% also, it imports about 75% from the total of Egypt's exports and imports from the Great Arab free trade Agreement during the period (2005-2009).

$$\text{In } Y_{ij} = \beta_o + \beta_1 \; I_n \; PcGDP - \beta_2 \; I_n Pc\,GDP_j + \beta_3 \; I_n \; DIST_{ij} + \xi_{ij}$$

$$\text{In } Y_{ij} = \beta_o + \beta_1 \; In \; Pc \; GDP_1 - \beta_2 \; In \; Pc \; GDP_j + \sum_{J=1}^{s} a_{j-y} D_j + \xi_{ij}$$

Where

Y_{ij} = Egypt's real exports and imports to or from the country (j) in Million Dollar

$Pc\ GDP_i$ = per- capita from the real local production total in Egypt (i) in thousand Dollar.

$Pc\ GDP_j$ = per – capita from the real local production total in the country (j) in thousand Dollar.

$Dist_{ij}$ = the Geographic distance between Egypt (i) and each country (j) in Kilometer.

D_{jt} = Dummy variable for the country (j).

The first model reflects the individual impact of the population of every country on Egyptian exports and imports of and contains variables of per-individual from the total local production of Egypt and the rest counties, also, the geographic distance between Egypt and each country.

Also the second model reflects the estimation of the same first model with inserting some dummy variables.

The estimation results of the gravity model for exports:

The equation (1) in Table (3) pointed to the gravity model of exports, from it, we conclude that the per-individual from total local production for each of Egypt and the other countries of the Great Arab free trade Agreement and the geographic distance between Egypt and each country, that explains about 80.2% of the variable changes in the

exports of Egypt to these countries, while the remained changes owed to other variables that are not measured in the model as for a definition factor measure, the model of gravity was signified at the level of 0.01 as for the "F-test".

The results show that increasing of per-capita from the total local production in Egypt by 1.0% lead to increasing of Exports of Egypt to the member Great Arab free trade Agreement by a percentage of 7.25% while it occurred that the per-capita from the total local production in each country of these countries by 1.0% lead to increase of the Egyptian exports to them by a percentage of 0.32%, also, it occurred that the increase the geographic distance between Egypt and each country by 1.0% lead to decrease in Egyptian exports by a percentage of 0.58% , that with the constancy of the other remained factors at a certain level.

Table (3): The results of estimation the gravity model for the exports and imports of Egypt with the grand Arab free trade Area during the period (1995-2009)

Constant var.	Exports Model (1)	Imports Model (2)
Constant/limit	-44.37 (-11.60)**	-45.34 (-6.49)**
Per-Individual from the total local production in Egypt.	7.25 (14.84)**	6.84 (7.31)**
Per- Individual from the total local production in GAFTA members.	0.32 (1.99)*	-66 (2.81)**
Geographic distance	-0.58 (-2.05)*	-0.49 (-1.67)***
N R^2 F-Test	150 0.820 (263.2)**	150 0.768 (73.4)**

Where: The numbers between brackets and under the factors of regression point to the values of the calculated (t).
-(*), (**), (***) point to the significance at the level of 0.05, 0.01, 0.10 squinty.
-N: Number of scenes, R^2: factor of definition, f-test: the value of the calculated f.
Source: Gathered & Calculated from the data of the text (1), (6), (17), (19).

The equation (1) mentioned in table (4) to the gravity modal of exports, to estimate the individual compact for each country on the Egyptian exports from this it occur that the per-capita from the total local production for each of Egypt and the member countries of the Great Arab free trade Agreement in the model and the geographic distance between Egypt and each countries of them explains about 87.5% of the changes in Egypt's exports to these countries while the remained changes is owed to other factors not measured in the model as the factor of definition, the gravity model is significant statistically at the level of 0.01% as for the F-Test the results shows that the increase of per-individual from the total local production of Egypt by 1.0% lead to increase Egypt's exports to the countries of the Great Arab free trade Agreement by a percentage of 5.74% , it also occurred that the increase of the per-individual form the total local in each of these countries by a percentage of 1.0% lead to the increase of Egypt's exports to the countries of the Great Arab free trade Agreement by a percentage of 2.12% while it occurred that the increase the geographic distance between Egypt and each country by 1.0% lead to decrease Egypt's exports by percentage of 0.32%, this was readied with the constancy of other factors at a certain level. The same results was showed by equation (2) in Table (4), that the value of "Group F-Test" to measure the individual impact for each country on the Egyptian exports was reached about (29.5), this was greater than the tabled value, thus rejecting the privative hypothesis and the acceptance of the alternative hypothesis that explains the existence of the impact of the individuals of these countries on the Egypt's exports, where , it showed that Sudan and Syria respond to the demand on Egypt's exports when the level of income of its individual by a percentage 0.62% and 0.51% it sequence in also occurred that the most important countries which were responsible.

The results of estimating the "gravity Model" for the imports:

The equation (2) in table (3) points to the "gravity Model" to measure the individual impact for each country on Egyptian imports, it occurs from it that per-capita from the total local production for each Egypt and the countries member of the grand Arab free trade area. In the model and the geographic distance between Egypt and each country of them, explain about 76.8% of the changes in the imports of Egypt for these countries while the remained changes refers to other factors not measured in the model as for the factor of

definition the "Gravity Model" was statistically signified at the level (0.01) as for the F-Test".

The results show that the increasing of per-capita from the total local production in Egypt by 1.0% lead to increase of Egyptian imports from the members of the Great Arab free trade Agreement by a percentage of 6.84% while it was occurred that when the per-individuals from the total local production in each of these countries increase by 1.0% lead to Egyptian imports increase by a percentage of 0.66% from them, also it occurred that the increase the geographic between Egypt and every country by 1.0% lead to decrease Egyptian imports by a percentage of 0.49% with constancy of other factors at a certain level.

Table (4): The results of estimation of the Gravity Model in measuring the impact of the countries of the Grand Arab free trade Area on the Egyptian foreign trade during the period (1995-2009)

Constant Var	(1) Exports Model	(2) Imports Model
Constant/limit	-48.83 (-14.54)**	-44.75 (-6.51)**
Per-Individual from the total local production in Egypt.	5.74 (9.00)**	7.43 (6.21)**
Per-Individual from the Total local production in (GAFT) members.	2.12 (3.93)**	0.05 (3.26)**
Geographic distance	-0.32 (-2.41)*	-0.51 (-1.99)*
The impact of each country		
Algeria	-1.57 (-3.97)**	1.48 (1.88)***
United Emirates	-4.64 (-3.35)**	1.96 (3.80)**
Kuwait	-5.50 (-4.36)**	1.61 (2.72)**
Lebanon	-0.04 (-2.17)*	0.58 (1.83)***
Libya	-3.10 (-3.20)**	1.68 (3.98)**
Saudi Arabia	-2.14 (-2.31)	3.73 (2.27)*
Sudan	0.62 (2.42)*	0.45 (2.08)*
Syria	0.51 (1.99)*	0.81 (2.37)*
N R^2 F-test Group F test	150 0.875 400.3 (29.5)**	150 0.748 121.1 (15.3)**

-N: Number of scenes, R2: factor of definition, F=test: the value of the calculated (F)
- Group F. test: The test of country impact.
- Source: Gathered & Calculated from the data of the test (1), (6) (17), (19)

of the decreasing of Egypt's exports in case of the raise of the individual income, are, Kuwait, United Emirates, Libya and Saudi Arabia, that shared in decreasing Egypt's exports by a percentages of 5.5%, 4.64% , 3.10% and 2.14% squinty.

The equation (2) in tab le (4) points to the "gravity Model" to measure the individual impact for each country on Egyptian imports it occurs from it that per-capita from the total local production for each Egypt and the countries member of the Great Arab free trade Agreement in the model and the geographic distance between Egypt and each country of them explains about 74.8% of the changes happened in Egyptian imports to these countries, while the remained changes to other factors that are not measured in the model as the factor of definition, the gravity model" was statistically signified at the level (0.01) as for the F-Test" the results explain that the increase of the Per-capita from the total local

production in Egypt by 1.0% lead to the increase in Egyptian imports from the country member of the Great Arab free trade Agreement by the percentage of 7.43% it also occurred that the increase of the per-capita from the total local production in each country of them by 1.0% lead to the increase in the Egyptian imports from the countries of the Great Arab free trade Agreement by a percentage of 0.05% while it occurred that the increase in the geographic distance between Egypt and each country by 1.5% lead to the decrease of Egyptian imports by a percentage by 0.51% , with the constancy the other remained factors at a certain level .

The same results of equation (2) in Table (4) that the value of "group F-Test" to measure the individual for each country on Egyptian imports, reached about (15.3) which is greater than the value in table, although we must refuse the hypothesis of privation and accept the alterative hypothesis which shows the existence of an impact of the individuals of these countries on Egyptian imports, where it explain the respond of Egypt to increase its imports from Saudi Arabia, Emirates , Libya and Kuwait by percentages of 3.73%, 1.95%, 1.68 and 1.61 squinty.

Recommendations:

1. Developing the Mutual fundamental projects between the members of the Great Arab free trade Agreement.
2. Improving the transportations between Egypt and countries of the Great Arab free trade Agreement, especially the two neighboring member countries, Libya and Sudan.
3. Exports of Egyptian goods and services take account of the consumer Arab taste and quality requirements through studying the inside markets member countries of the Great Arab free trade Agreement.
4. Establishing a mutual area for investment between the members of the Great Arab free trade Agreement.
5. Removing the obstacles and the non –custom strains that disrupt achieving and activating the agreement and obligate all the member countries to follow rules and discussions of the agreement of what deal with liberation of the custom and concealing the non-custom strains.
6. Elasticity inactivating the cleanses of the agreement, taking in consideration the less-developing countries and their economic status.
7. Limitation of prescription of exceptions of the custom liberation to some member countries.
8. Impelling the Arab countries that are not members in the agreement to complete their procedures of joining the Great Arab Free Trade Agreement.
9. Facilitative the procedures of graining visas among the members.

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Halophytic Plants for Phytoremediation of Heavy Metals Contaminated Soil

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Abstract: Using of halophyte species for heavy metal remediation is of particular interest since these plants are naturally present in soils characterized by excess of toxic ions, mainly sodium and chloride. In a pot experiment, three halophyte species *viz. Sporobolus virginicus*, *Spartina patens* (monocotyledons) and *Atriplex nammularia* (dicotyledon) were grown under two levels of heavy metals: 0 level and combinations of 25 mg Zn + 25 mg Cu + 25 mg Ni/kg soil. The three species demonstrated high tolerance to heavy metal salts in terms of dry matter production. *Sporobolus virginicus* reduced Zn, Cu, and Ni from soil to reach a level not significantly different from that of the untreated control soil. Similarly, *Spartina patens* significantly reduced levels of Zn and Cu but not Ni. *Atriplex nummularia* failed to reduced Zn, Cu and Ni during the experimental period (two months). Only *Sporobolus virginicus* succeeded to translocate Zn and Cu from soil to the aerial parts of the plant. The accumulation efficiency of Zn and Cu in aerial parts of *Sporobolus virginicus* was three and two folds higher than *Spartina patens* and around six and three times more than *Atriplex nammularia* for both metals, respectively.
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Key words: Spartina *patens, Sporobolus virginicus, Atriplex nummularia*, Zn, Cu and Ni, Phytoremediation

1. Introduction:

The contamination of soil by heavy metals is one of the most serious environmental problems and has significant implications for human health. Some industrial activities and agricultural practices increase their level in the substrate, and the possible introduction of the elements in the food chain is an increasing human health concern (**Cakmak *et al.*, 2000).** Engineering industrial techniques may efficiency be used to clean up contaminated soils but most of them require sophisticated technology and are therefore expensive and suitable only for small polluted areas **(Lutts *et al.*, 2004).** Furthermore, these technologies are not only costly, but they also cause soil disturbances and they are not readily accepted by the general public **(Gardea-Torresdey *et al.*, 2004 and Manousaki *et al.*, 2007).** Phytoremediation has been highlighted as an alternative technique to traditional methodologies, for the removal of heavy metals from soil. Two approaches have been generally proposed for the phytoremediation of heavy metals. The first one is using of natural hyperaccumulator plants with exceptional metal-accumulating capacity. However, the hyperaccumulator plants usually accumulate only a specific element, tended to grow slowly and to have a low biomass. Many of them are not suitable to be used as phytoextraction in the field. While the second is utilizing of high-biomass plants whereas, relatively high amounts of the metal accumulated by plants is often translocated from the root to the more easily harvestable shoots **(Chen *et al.*, 2004 and Manousaki *et al.*, 2007).** Disposal options include pyrolysis, composting, and compaction as pretreatment steps in order to reduce the volume of plant material and incineration, ashing, liquid extraction, or direct disposal-since plants are easier to dispose of than soil as final disposal.

Zinc, copper and nickel are of significant importance because their excessive amounts in the soil can arise not only due to human economic activity but also because of nature soil forming processes. In fact, these heavy metals occur in some parent material of soils in high concentrations. Thus, Cu content in soil was shown to vary 1000-fold (from 2 to 2000 mg/kg soil); Zn more than 4000 fold (from 25 to more than 10000 mg/kg soil **(Kholodova *et al.*, 2005).** Therefore, searching for plants capable of accumulation the high concentrations of these metals in their shoots is of interest in respect to phytoremediation technology. Growing of such plants permit natural soil cleaning up from excess heavy metals, thus helping other, less tolerant plant species to inhabit the region and consequently soil can be restored for farming **(Lombi *et al.*, 2001; Gleba *et al.*, 1999; Lasat, 2002 and Zhulidov *et al.*, 2002).**

Halophytes are the plants naturally grow in high salt affected soils. They have developed different strategies to survive and complete their life cycles, sometimes in very high salinity level reached over seawater salinity level.

Sporobolus virginicus, *Spartina patens* and *Atrriplex nummularia* are halophyte species remove excess salt from the soil by various strategies. The three plants have salt glands through which salt is excreted from their leaves. This capability to uptake

salt from soil and translocated from root into aboveground shoot without, in most cases, a significant decrease of shoot biomass production is an advantage not available in conventional crops. Moreover, halophytes are found usually in arid and semi arid regions and can grow on land of marginal quality which allows their use for phytoremediation of soils with low fertility and poor soil structure, resulting in low operating costs.

The aim of this study was to investigate the possibility of using some halophytic plants as phytoremediation as well as its efficiency under Egyptian conditions.

2. Materials and Methods

To investigate the possibility of using some halophytic plants as phytoremediation a pot experiment was carried out in greenhouse at the Faculty of Agriculture, Ain Shams University, Cairo, Egypt. *Sporobolus virginicus* (L.) Kunth (smyrna) and *Spartina patents* (Aiton) Muhl. were introduced from Delaware, U.S.A. Rhizomes of these grasses were first transplanted in pots under greenhouse at the National Research center Cairo, Egypt. Some pots of these grasses were again transplanted into greenhouse of Agricultural Botany Dept. at Faculty of Agriculture, Ain Shams University. Rhizome cuttings 5-8 cm in length were rooted in tap water. *Atriplex nummularia* Lindl seedling was obtained from Desert Research Center, Cairo, Egypt and planted in the greenhouse of Agricultural Botany Department on October 2006. Two years later, stem cuttings 30 cm length were taken and rooted in tap water. All rooted cuttings were planted in pots on September 1st 2008.

Factorial experiment was conducted in randomized complete block design including 3 halophytic species and 2 levels of heavy metals (without and with 25 mg Zn + 25 mg Cu + 25 mg Ni/kg soil) with 5 replicates. The combination of metals was based on Zn equivalent of 260 mg/kg (the level of Zn caused toxic effect for most plants) in which Zn was replaced equally with Cu or Ni assuming that Cu was twice as toxic and Ni was eight times as toxic as Zn **(Davies, 1980)**. So that the amounts of Zn, Cu and Ni used were 275 mg/kg as Zn equivalent. Soil sample was characterized by pH = 7.8, ECe = 2.5 dS/m, $CaCO_3$ = 3.2 %, organic matter = 0.02 %, Clay = 2 %, Silt = 3 % and Sand = 95 %. The soil texture grade was sand (Typic Torripsament). Each pot was filled with 5 kg sandy soil. Soil was well mixed with 1.88g ammonium sulfate (equal 190 kg N/ha), 1.39g potassium phosphate (equal 190kg K and 150kg P/ha). Pots of treatment with heavy metal were well mixed with 0.548g $ZnSO_4.7H_2O$ + 0.265g $CuCl_2$ + 0.506g $NiCl_2.6H_2O$. Pots were watered to keep moisture content approximately at 60% of water holding capacity. Eight weeks after planting date the plants were harvested and soil samples were taken from each pot, then plant and soil samples were air dried and kept in plastic bags for analysis.

All soil chemical properties were determined according to **Page *et al.* (1982)**. Soil pH was determined in 1:2.5 soil, water suspension. Calcium carbonate was determined with calcimeter. Available Zn, Cu and Ni were extracted by DTPA extractant and then determined using atomic absorption spectrophotometer (Varian Spetra AA20, Victoria, Australia).

Dried plant samples were wet ashed with the ternary acid mixture, HNO_3, $HClO_4$, H_2SO_4. Plant contents of Zn, Cu and Ni were determined as mentioned above.

All parameters of soils and plants were analyzed statically by multiple factor analysis of variance in randomized complete block design using Tukey's multiple range test of significance at 5% level as described by **Steel and Torrie (1980)**.

3. Results

Data presented in Table (1) and illustrated in Fig. (1) showed that the heavy metal salts had no significant effect on aboveground dry matter production of the three halophyte species used in this study. On the other hand, *Sporobolus virginicus* recorded the highest significant value of areal biomass followed by *Spartina patens*. While *Atriplex nummularia* produced the least significant value of shoot yield during the experimental period.

The total removal of Zn, Cu and Ni from the contaminated soil by plants is presented in Table (1) and Figs. (2-4). Data indicated that *Sporobolus* plants remediate soluble Zn, Cu and Ni salts from treated soil causing significant reduction of the metals level to be statistically equal with untreated control soil. However, *Sporobolus* plants reduced level of soluble Ni in contaminated soil yet less than that detected for Zn and Cu. *Spartina patens* came descendingly after *Sporobolus virginicus* and significantly reduced level of Zn and Cu in contaminated soil to be similar with untreated soil. In spite of obviously reduction of soluble Ni in treated soil by *Spartina* plants, but Ni level still significant higher than that found in untreated soil. *Atriplex nummularia* failed to achieve the same efficiency of the other two plants. The level of Zn, Cu and Ni in contaminated soil exhibited the highest significant values by using *Atriplex nummularia* compared with either *Sporobolus* or *Spartina* plants.

Almost the same pattern was found by examining the heavy metals concentration and uptake

by the plant shoots as presented in Figs (5-10). Here again, the highest accumulation levels of Zn, Cu and Ni were achieved by *Sporobolus virginicus* to record 48.4, 8.7 and 28.3 g.Kg^{-1}dw, followed by *Spartina patens* (16.4, 4.4 and 28.7 g.Kg^{-1}dw). Meanwhile, *Atriplex nummularia* accumulated the lowest values 8.5, 2.5 and 23.0 g.g^{-1}dw for Zn, Cu and Ni, respectively. However, only Zn and Cu achieved a higher significant accumulation level by *Sporobolus* plants above all other treatments. No significant differences were detected either for *Spartina* or *Atriplex* plants on concentration or uptake of heavy metals in their aerial part above untreated ones.

Table 1. Analysis of variance and multiple range test of Zn, Cu and Ni available in soil, concentrations and uptake by different plants untreated and treated with heavy metals.

Source of variance		Dry weight	Soil available			Plant content			Plant uptake		
			Zn	Cu	Ni	Zn	Cu	Ni	Zn	Cu	Ni
Analysis of variance											
Type of plant		***	***	***	***	***	*	NS	***	***	***
Heavy metals		NS	***	***	***	***	**	**	***	***	**
Interactions of plants X metals		NS	***	***	**	***	**	NS	***	***	NS
Tukey's Multiple range test											
Main effect											
Type of plant	*Spartina*	B	B	B	B	B	A	NS	B	B	B
	Sporobolus	A	B	B	B	A	A	NS	A	A	A
	Atriplex	C	A	A	A	B	A	NS	B	B	B
Heavy metals											
	without	NS	B	B	B	B	B	B	B	B	B
	With	NS	A	A	A	A	A	A	A	A	A
Interactions											
Spartina Without heavy metals		NS	B	B	C	B	B	NS	BC	B	NS
Spartina with heavy metals		NS	B	B	B	B	B	NS	BC	B	NS
Sporobolus Without heavy metals		NS	B	B	C	B	B	NS	B	B	NS
Sporobolus with heavy metals		NS	B	B	BC	A	A	NS	A	A	NS
Atriplex Without heavy metals		NS	B	B	BC	B	B	NS	C	B	NS
Atriplex With heavy metals		NS	A	A	A	B	B	NS	C	B	NS

*,** and *** significant effect at $P < 0.05$, 0.01 and 0.001, respectively, NS = not significant.
The same streaks within factors are not different but a value A > B > C …etc at 5% level.

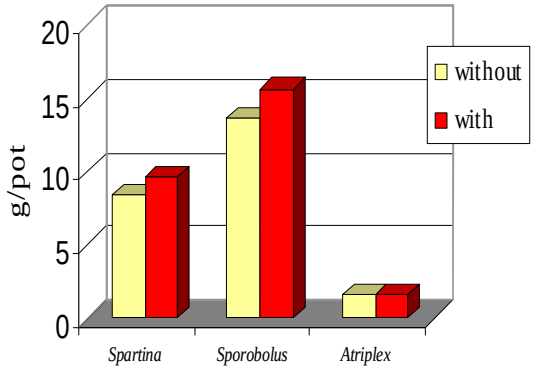

Fig. 1. Effect of heavy metals treatment on the dry weights of different halophyte plants.

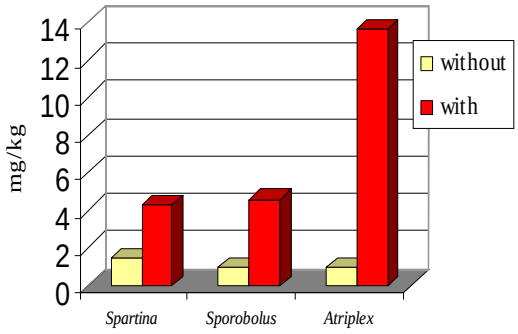

Fig. 2. Effect of different plants on the available Zn in the untreated and treated soil with heavy metals.

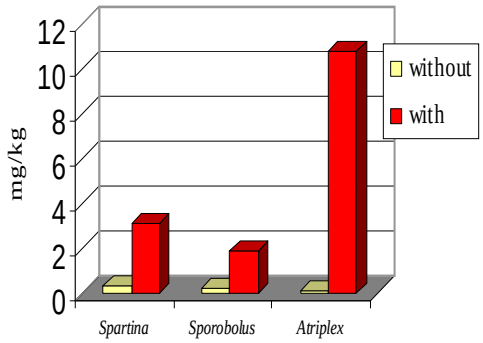

Fig. 3. Effect of different plants on the available Cu in the untreated and treated soil with heavy metals.

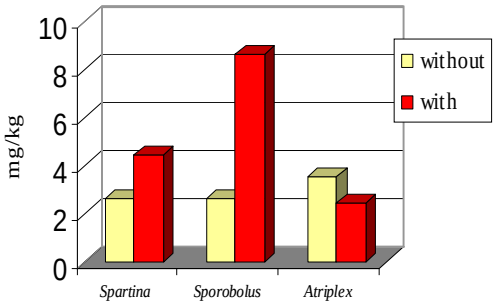

Fig. 6. Copper concentration in different plants grown on untreated and treated soil with heavy metals.

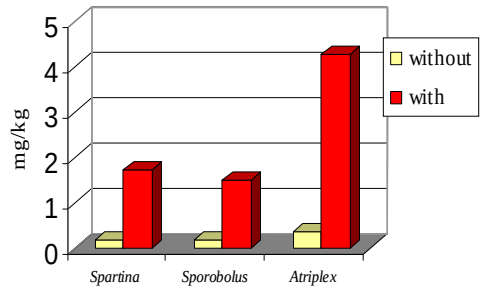

Fig. 4. Effect of different plants on the available Ni in the untreated and treated soil with heavy metals.

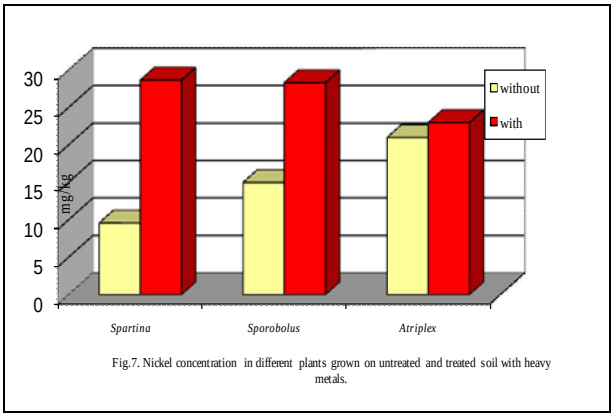

Fig.7. Nickel concentration in different plants grown on untreated and treated soil with heavy metals.

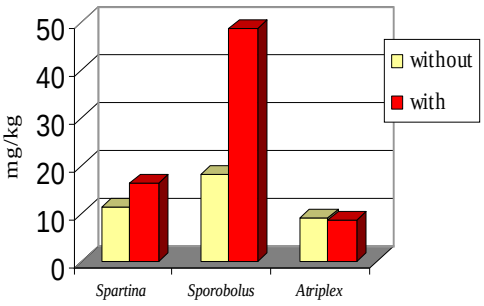

Fig. 5. Zinc concentration in different plants grown on untreated and treated soil with heavy metals.

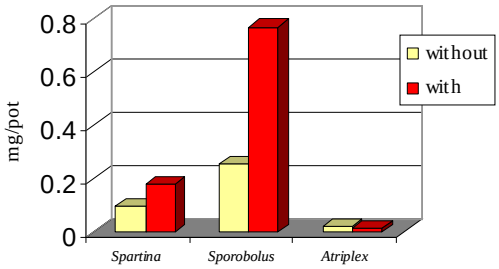

Fig. 8. Zinc uptake by different plants grown on untreated and treated soil with heavy metals.

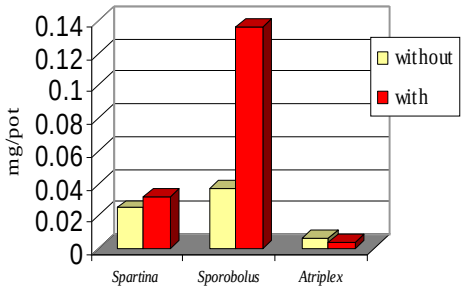

Fig. 9. Copper uptake by different plants grown on untreated and treated soil with heavy metals.

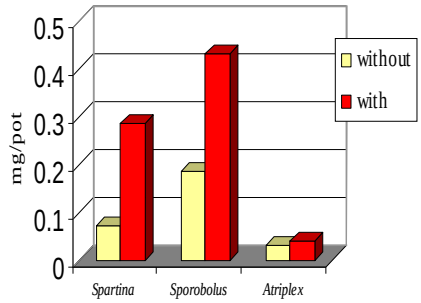

Fig. 10. Nickel uptake by different plants grown on untreated and treated soil with heavy metals.

4. Discussion

The present work showed that the halophytic species *Sporobolus virginicus*, *Spartina patens* (monocotyledons) and *Atriplex nummularia* (dicotyledon) demonstrated a high tolerance to Zn, Cu and Ni salts. Also they accumulated these elements without showing any significant decrease in terms of dry biomass production. On the other hand, there were significant differences in the aboveground biomass production among the three species, the highest significant biomass values were found in *Sporobolus virginicus* followed by *Spartina pa*tens but *Atriplex nummularia* recorded the lowest value during the experimental period. In this respect, **Kholodova *et al.* (2005)** reported that seeds and young seedlings of the halophyte *Mesembryanthemum crystallinum* demonstrated a high tolerance of heavy metals salts. They added that, the halophyte *Mesembryanthemum crystallinum* could grow at $CuSO_4$ concentrations 200-fold and $ZnSO_4$ concentrations 800-fold higher than those in standard nutrient medium providing for normal development. On the other hand, the use of halophyte species for heavy metal remediation is of particular interest because these plants are naturally present in environments characterized by an excess of toxic ions, mainly sodium and chloride. Several studies demonstrated that some tolerance mechanisms operating at the whole plant level are not always specific to sodium but also for other toxic elements such as copper, zinc or cadmium **(Hagemeyer and Waisel, 1988; Neumann *et al.*, 1995; McFarlane and Burchett, 1999; Lutts *et al.*, 2004; Reboreda and Cacador, 2007 and Manousaki *et al.*, 2007).** Drought and/or salinity resistance mechanisms in halophyte species may indirectly contribute to heavy metals tolerance, since high level of heavy metals salts are responsible for secondary water stress in plant **(Poschenrieder *et al.*, 1989)**.

Heavy metals stress also had a limited effect on the concentration of other elements. A decrease in calcium may be partially explained by the fact that divalent cations can translocate in the plant through nonselective calcium channels**.** Also, a strong inhibition of photosynthesis may result from iron deficiency in Cd-treated plants or iron transportation from root to shoot was reduced under zinc-treated plants **(Lutts *et al.*, 2004).**

On the other hand, the three halophytic plants used in the present study have salt glands. In this respect, **Freitas and Breckle (1993)** reported that many halophyte species are able not long to accumulate high amount of Na in trichomes covering the leaf surface but also other elements may also accumulate when present in excess. Trichomes accumulate both Zn and Cd in the resistant species *Arabidopsis halleri* (L.) **(Küpper *et al.*, 2000)**, and Cd in the halophyte plant *Tamarix aphylla* (L.) **(Hagemeyer and Waisel, 1988)**. Specific over expressions of a gene coding for a metallothionein (MTZ) has been reported in trichomes of various halophyte species **(Folcy and Singh, 1994 and Garcia-Hernandez *et al.*, 1998),** there were constituted important sites for toxic metal accumulation **(Lutts *et al.*, 2004)**. In the present study, the three halophytes used have the ability to excrete the excess NaCl under salt effect through salt glands and trichomes covering their leaf surface. Therefore, it may be suggested that in Cu and Ni salts were excreted through trichomes and salt glands to leaf surface then washed out mainly by dew. This gave the reason for sharply reduction of heavy metals level from the contaminated soil without significant accumulation in plant shoots in particularly for *Spartina* and also for Ni concentration in both *Sporobolus* and *Spartina* plants. Also, gave the answer why these plants are tolerant for the high concentration of heavy metals. On the other hand, the three halophytes are C_4 plants characteristic by fast growing and high biomass in short time than other conventional crop particularly without fertilizer

supply. In this respect **Chen *et al.* (2004)** mentioned that plants used for phytoextraction should be tolerant to the high concentrations of metals without significant inhibitions of growth or reduction in biomass production.

In light of recently work it appears that plants used for phytoextraction must first become well developed and established in contaminated soil.

Sporobolus and *Spartina* plants responded faster by transplanting and grown better than *Atriplex*. That might be due to the type of root system, whereas the monocytedonous *Sporobolus* and *Spartina* plants characterized by adventurous root system which grown faster after transplanting than tap root of *Atriplex*. *Sporobolus* reached its maximum aboveground biomass after two months. Meanwhile, the same period was not enough for *Atriplex* plant to reach its maximum aerial biomass. The aim of phytoremediation is to reduce the level of metals in the contaminated soil to acceptable levels within a reasonable time frame. In light of recently work, it appears that *Sporobolus* plant has the greatest efficiency for phytoremediation of Zn, Cu and Ni salts from contaminated soils.

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Effects of Chronic Exposure to Static Electromagnetic Field on Certain Histological Aspects of the Spleen and Some Haematological Parameters in Albino Rats

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Abstract: Over the past few years, our environment has become seething electromagnetic smog that bombards our bodies every second of every day. Because electro-magnetic fields are invisible, we do not even realize they are there, although they are battering us mercilessly. Special attention has been given to the biological effects of magnetic fields. Thirty six male albino rats *(Rattus norvegicus*) were utilized in the present work to study the effects of static magnetic field (SMF) equaling 2 ml tesla on the spleen and some haematological parameters. Magnetic exposure was applied for ١0 minutes for 3 days per week for two weeks. One day following magnetic exposure, the spleen showed congestion in the splenic sinusoids accompanied with thickening of the splenic capsule. A significant increase in the white blood cells and blood platelets was accompanied by enlargement of the white pulp were detected. Seven days following magnetic exposure, an increase of haemoglobin concentration; haematocrit and red blood cells was recorded accompanied with a highly significant decrease in blood iron. Later on, such increase was followed by a significant decrease in most haematological parameters after fifteen days of magnetic exposure. Hemosiderin granules were observed in the dilated splenic sinusoids at the areas of congestion. The splenic tissues and the haematological parameters appeared almost normal and manifested a tendency towards recovery after thirty days following magnetic exposure.

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Keyword: Static Electromagnetic Field Histological Aspects Spleen Haematological Parameters in Albino Rats

Introduction:

During the past few decades, there was a growing concern about the increase in invisible environmental pollution due to the emission of electromagnetic waves **(Tonini et al., 2001; Chakeres & de Vocht 2005).** Numerous epidemiological studies have failed to find a correlation between magnetic field at different intensities and the appearance of any particular pathological changes **(Reipert et al., 1997; Day, 1999; Schüz & Ahlbom, 2008;Calvente et al., 2010).**

Previous studies showed that electromagnetic fields induced changes in haematological parameters in mice, rats and humans **(High et al., 2000; Ali et al., 2003; Sihem et al., 2006; Hassan & Abdelkawi, 2010).**

Considerable evidence has been accumulated regarding the biological effects of static magnetic field focused on sources of exposure and interaction mechanisms **(Feychting, 2005; Rongen, 2005; Straume et al., 2008; Kundi et al., 2009).** It was reported that paramagnetic properties of iron storing organs make these organs more likely to be affacted by magnetic fields **(Gorczynska & Wegrzynowicz, 1991).**

Other researchers demonstrated the interaction of static electromagnetic field (EMF) with the immune system **(Thun-Battersby et al., 1999 ; Marino et al., 2000 ; Attia & Yehya, 2002 ; Johansson, 2009)**

The role of the spleen as a lymphatic organ storing iron in immune system and the increased application of magnetic field-generating equipment, stimulate the conduction of the present experimental study, targeting chronic exposure of the spleen to a moderate static magnetic field and some of haematological parameters in albino rats.

Material and Methods

Animals:

Thirty male albino rats *(Rattus norvegicus*), each weighs 120 $^{\pm}$ 10 grams were utilized in the present study. The animals were housed in plastic cages to avoid any metallic interaction and were kept under similar normal laboratory conditions during the period of the experimental study.

The animals were divided into two groups:

Group I : This group included six rats used as control (unexposed animals).

Group II: This group included thirty rats exposed individually to constant electric magnetic field (direct current, DC) with flux density equal to 2 ml tesla.

EMF Exposure:

The exposure was applied for ١0 minutes/ day, 3 days / week, for 2 weeks. The EMF was generated

by applying an electric current to the coil of artificial EMF apparatus constructed in the Department of Physics, Jubail Faculty of Education for Girls, Kingdom of Saudi Arabia. The animals were kept in a perforated plastic box, placed in the coil chamber of the apparatus. Then, a horizontal magnetic induction was applied to the whole animal body. The field strength was monitored with a gauss meter.

At the end of the experiment, all the animals were dissected and specimens of spleen were taken on 1, 7, 15, and 30 days post-irradiation. All the control animals and six animals of the second group were sacrificed after 1, 3,6,15 and 30 days following the exposure.

Histological methods:

Small spleen specimens were cut into small blocks, fixed in 10% neutral buffered formalin and processed up to paraffin pieces. Semi-serial sections of 6 µm were prepared and stained with Harris' hematoxylin and eosin (**Drury & Wallington 1980)** to illustrate the histological structure. Spleen specimens also stained for haemosiderin by **Perl's Prussian blue reaction technique (1867)** for detection of haemosiderin iron.

Haematological and Blood chemical methods:

At the end of the experiment the blood samples were collected (0.5 ml approximately/ sample) from the supra-orbital venous plexus of rats using heparinized syringes into two tubes. The first tube contained heparin as anticoagulant. The heparinized blood was used for RBC, haematocrit, haemoglobin, WBC and platelets analysis, using standard methods **(Feldman et al., 2000).**

The blood sample in the other tube was left for a short time to allow clotting. Clear serum samples were obtained by centrifugation at 3000 r.p.m. for 20 min and then kept at -20°C prior to biochemical analysis.

A serum level of iron was measured using Stanbio serum iron liquicolour commercial Kits, (Procepdure No. 0370) according to the method by **Weissman &Leggi (1974).**

Statistical Analysis:

The data was present as the mean ± SD for each correlation was calculated using Microsoft Excel.

Results

I. The control animals:

The spleen is surrounded by a fibrous connective tissue capsule interspersed with smooth muscle fibres . Irregular spaced trabeculae of smooth muscle and fibroelastic tissue emanate from the splenic capsule into the splenic parenchyma containing blood and lymph vessels.

The parenchyma of the spleen is termed the pulp. Most of the pulp is soft red. It consists of large, irregular, thin-walled blood vessels, splenic sinusoids, interposed between sheets of thin connective tissues and splenic cords. The splenic cords are the masses of cells in between the sinusoids. They contain a lot of erythrocytes (RBCs) and some other cell types as macrophages and megakaryocytes.

Within the red pulp, small oval or/and rounded grayish blue stained areas represent the white pulps. They consist almost entirely of lymphocytes, in a peculiar association with the arterial blood supply **(Fig .1)**.

The splenic tissue reveals a scanty amount of blue stained haemosiderin granules that are diffused and distributed throughout the red pulp cords **(Fig. 2).**

II. The SMF-exposed animals:

1. One day post exposure to SMF:

The spleen had a thick connective tissue capsule, variable in its thickness at different areas. Numerous thick trabeculae extended from the splenic capsule inwards, branched and divided the spleen into numerous parts. There were multiple clear areas in the subcapsular spaces and few haemorrhagic areas as directed inwards. Within the red pulps, small foci of cellular necrosis were observed and the splenic sinusoids were slightly dilated and congested with blood cells **(Fig.3)**.

Most of the white pulps were large and irregular in shape revealing cellular proliferation of homogeneous population of immunoplastic cells, and macrophages. Small foci of cellular lesions were observed scattering throughout the white pulps **(Fig .4).**

2. Seven days post exposure to SMF:

Numerous subcapsular clear spaces were found between the capsule and trabeculae . Few other ones containing haemolysed RBCs were observed between the splenic parenchyma **(Fig. 5)**.

The cells of the splenic cords revealed necrotic changes scattering throughout the red pulp. The splenic sinusoids were dilated contained some of erythrocytes or hemolysed blood. Numerous inflammatory cells and megakaryocytes were noted in the splenic parenchyma and sinusoids.

The white pulps were numerous, fragmented and appeared diffused in an irregular manner in the red pulp **(Fig. 6)**.

3. Fifteen days post exposure to SMF:

The splenic vasculature revealed progressive changes represented by congestion of the splenic sinuses and diffusion of haemorrhagic and hemolysed areas throughout the red pulps. Numerous inflammatory cells, macrophage and megakaryocytes were observed in the splenic parenchyma and sinusoids **(Fig.7).**

Some splenic white pulps suffered from lymphoid depletion and the others became hyperplastic.

Heavy iron blue pigments of hemosiderin granules were observed in the dilated red pulp spaces which were filled with hemolysed red blood cells **(Fig.8).**

4. Thirty days post the end of exposure to SMF:

The splenic tissues appeared almost normal and manifested a tendency towards recovery. Some significant signs towards complete vasculature and tissues recovery were observed in both red and white pulp as compared with the previous examined groups, where no areas of blood haemorrhage or haemolysis were observed.

Brown hemosiderin granules were seen in the cytoplasm of macrophages **(Fig .9).**

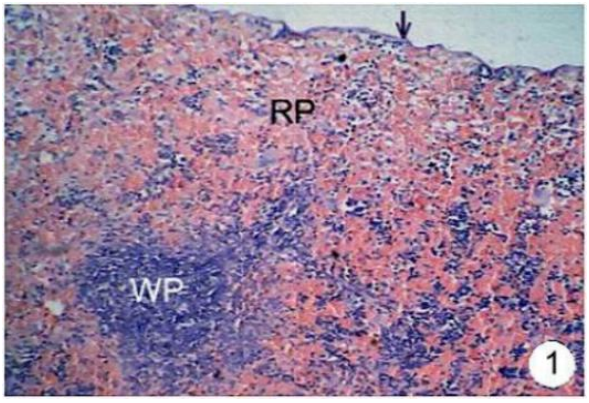


Fig. 1: A photomicrograph of a spleen section of control rat showing a fibrous connective tissue capsule surrounding the splenic pulp **(arrow).** Most of the pulp is a soft red pulp (RP) and some oval grayish blue-stained areas represent the white pulp (WP)**.** **(H&E stain X250)**

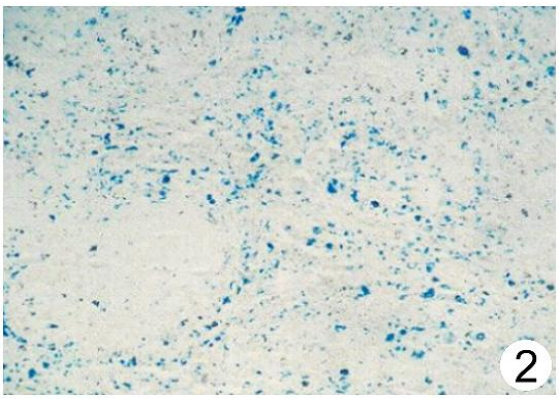


Fig. 2: A photomicrograph of a spleen section of control rat showing a splenic tissue revealing a scanty amount of blue-stained haemosiderin granules diffusely distributed throughout the red pulp cords**. (Perl's Prussian blue stain X400)**

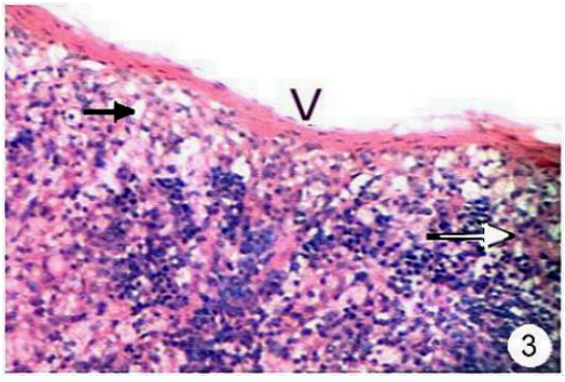


Fig. 3: A photomicrograph of a spleen section of rat after one day following the end of SMF exposure showing a thick connective tissue capsule **(arrowhead) ,** Multiple clear sinusoids in the subcapsular space**(black arrow)** and few haemorrhagic **(white arrow). (H&E stain X400**

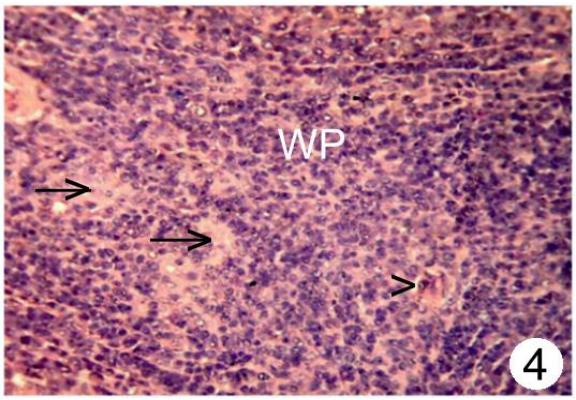


Fig. 4: A photomicrograph of a spleen section of rat after one day following the end of SMF exposure showing large and irregular white pulps (WP) revealing cellular proliferation of homogeneous population of immunoplastic cells, and macrophages **(arrowhead)** in addition to small foci of cellular lesions **(arrow). (H&E stain X400)**

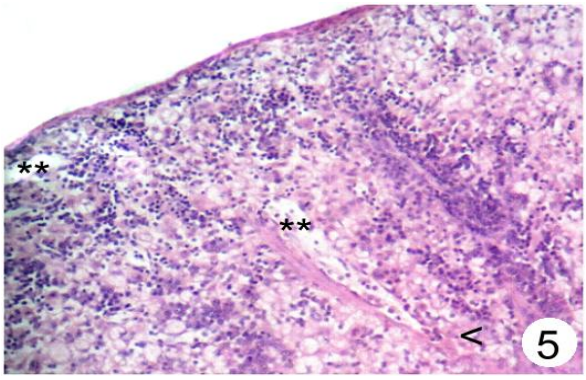


Fig. 5: A photomicrograph of a spleen section of rat after seven days following the end of SMF exposure showing numerous subcapsular clear spaces **(**)** and few other ones containing haemolysed RBCs **(arrowhead)** between splenic parenchyma. **(H&E stain X250)**

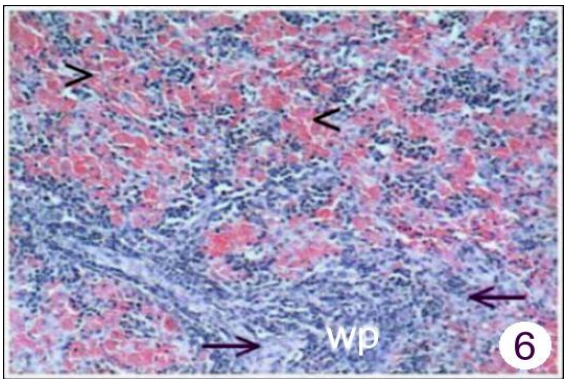


Fig. 6: A photomicrograph of a spleen section of rat after seven days following the end of SMF exposure showing dilated splenic sinusoids containing some of blood cells **(arrowhead),** or hemolysed blood. The white pulps **(WP) (WP)** are numerous, fragmented and appear diffused in an irregular manner throughout the red pulp. **(H&E Stain X400)**

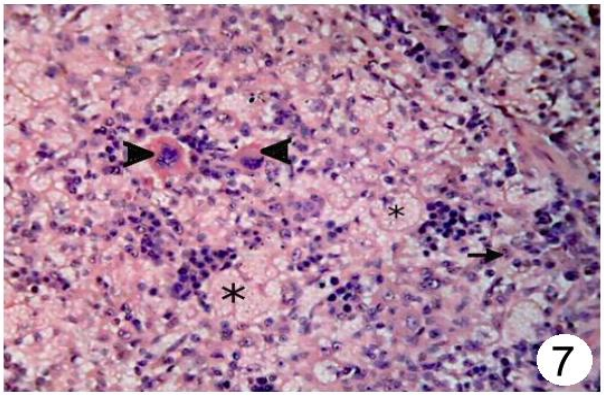


Fig. 7: A photomicrograph of a spleen section of rat after fifteen days following the end of SMF exposure showing haemorrhagic and haemolysed sinusoids diffused throughout the red pulps **(*),** some inflammatory cells, macrophage **(arrow)** and megakaryocytes **(arrowheads)** are observed in the splenic parenchyma**.** (**H&E Stain X400)**

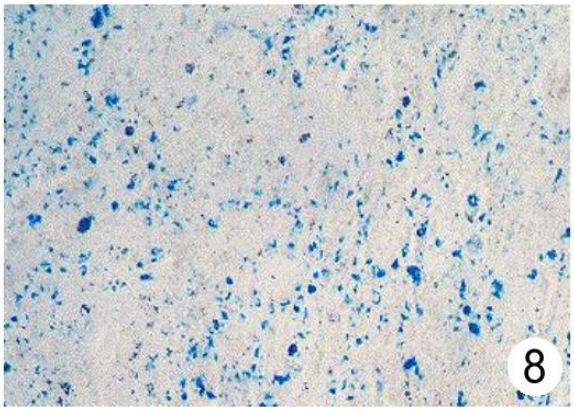


Fig. 8: A photomicrograph of a spleen section of rat after fifteen days following the end of SMF exposure showing heavy iron blue pigments of haemosiderin granules in the dilated red pulp spaces**. (Perl's Prussian blue method X400)**

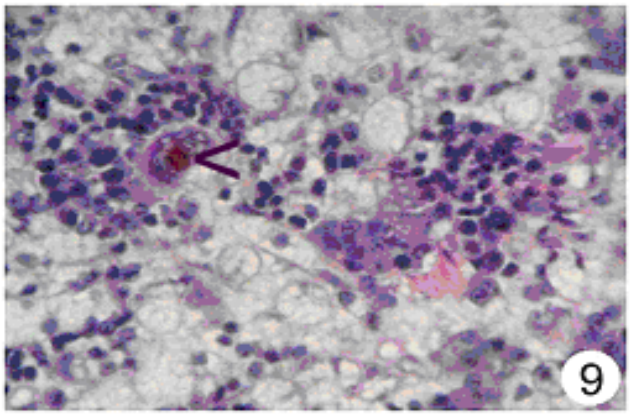


Fig. 9: A photomicrograph of a spleen section of rat after thirty days following the end of SMF exposure showing brown haemosiderin granules (arrowhead) in the cytoplasm of macrophages. **(H&E Stain X600)**

Haematological results;

Table (1): Effect of EMF on serum iron levels (µg/dl) of adult male rats

GROUP	Serum iron µg/dl (M±SE)
Control	153.44 ± 9. 56
One day	133. 49 ± 7.46*
7 days	118.49 ± 11.56**
15 days	173. 54 ± 17.46*
30 days	149. 34 ± 5.37

All data are Mean value± Standard error. N= 6 animals of each group.
* Significant at ($p < 0.05$). ** Highly significant at (< 0.01).

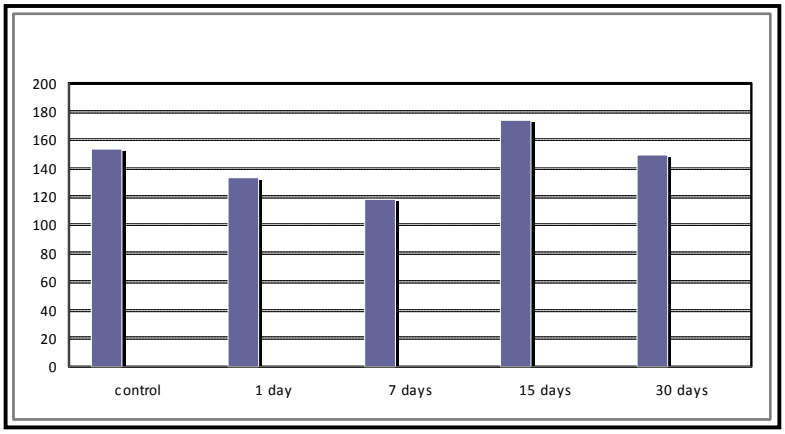


Table (2): Effect of EMF on red blood cells (RBCs) count (10^6/mm ^{3}) of adult male rats

GROUP	Red blood cells (RBCs) 10^6/mm ^{3} (M±SE)
Control	7.25 ± 0. 13
One day	7. 16 ± 0. 42
7 days	7. 89 ± 0. 29*
15 days	6.67± 0. 36*
30 days	7. 88 ± 0. 51

All data are Mean value± Standard error. N= 6 animals of each group.
* Significant at ($p < 0.05$). ** Highly significant at (< 0.01).

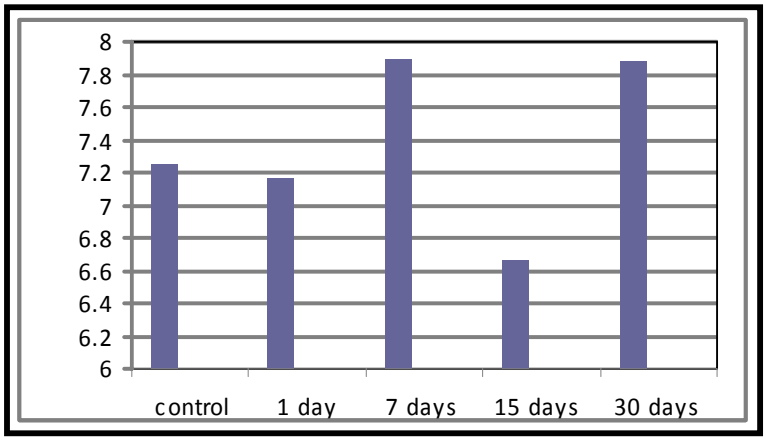


Table (3): Effect of EMF on haemoglobin levels (g/dl) of adult male rats Haemoglobin levels (g/dl) of adult male rats

GROUP	Haemoglobin levels (g/dl) (M±SE)
Control	11. 98 ± 0.25
One day	9. 34 ± 0.21*
7 days	13. 54 ± 0.46*
15 days	8. 95 ± 0. 39*
30 days	10. 55 ± 0. 43

All data are Mean value± Standard error. N= 6 animals of each group.
* Significant at (p < 0.05). ** Highly significant at (< 0.01).

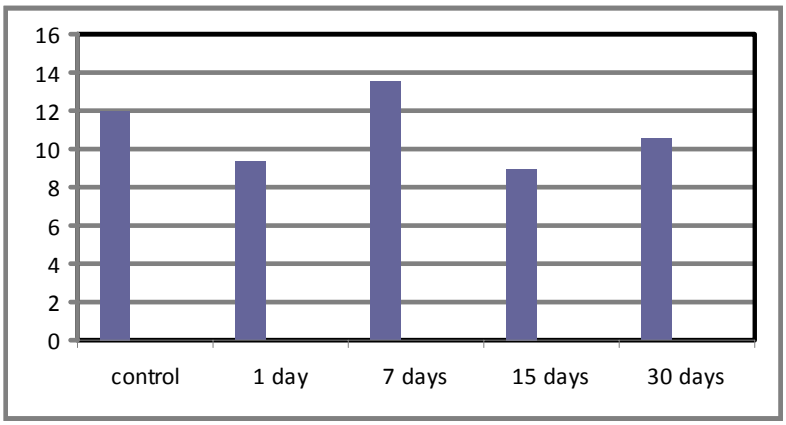


Table (4): Effect of EMF on haematocrit value % of adult male rats haematocrit value %

GROUP	Ht (%)
Control	36. 21 ± 0. 45
One day	31. 1 9 ± 0.26*
7 days	41. 54 ± 0. 76*
15 days	29. 54 ± 0.46**
30 days	37. 34 ± 0. 76

All data are Mean value± Standard error N= 6 animals of each group.
* Significant at (p < 0.05). ** Highly significant at (p < 0.01).

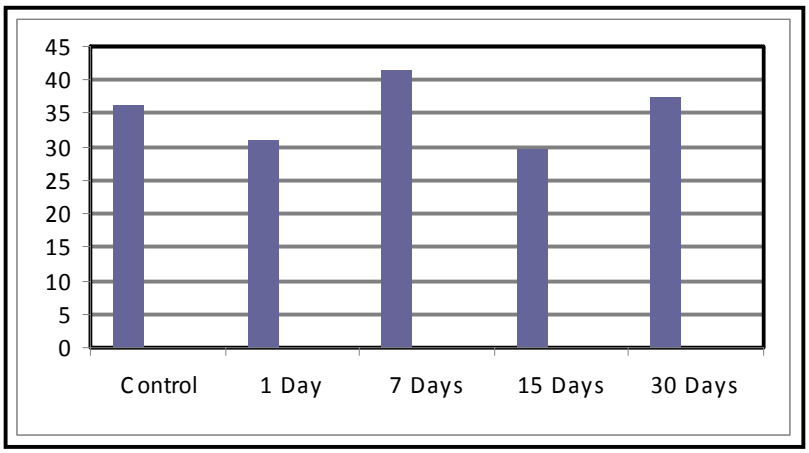


Table (5): Effect of EMF on white blood cells (WBCs) count (10^3/mm ^{3}) of adult male rats

GROUP	Blood platelets 103/mm 3 (M+SE)
Control	536.23 + 14.8
One day	628.17 +15.45*
7 days	694.45 + 35.65**
15 days	634.16 +12.73*
30 days	602.33+14.04

All data are Mean value± Standard error N= 6 animals of each group.
* Significant at ($p < 0.05$). ** Highly significant at ($p < 0.01$).

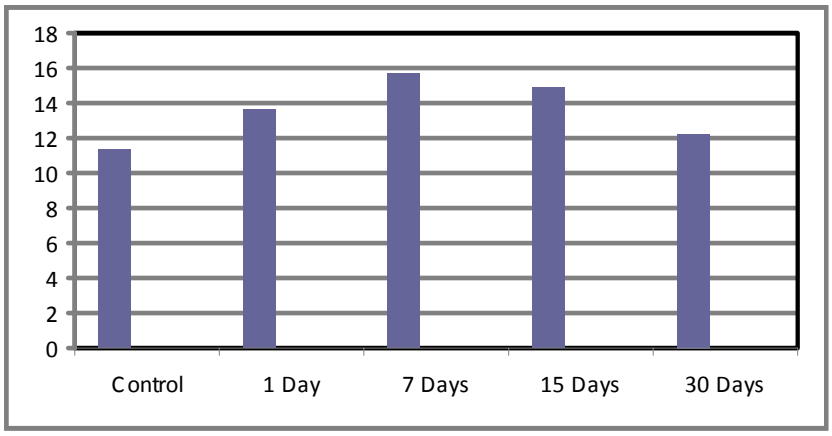
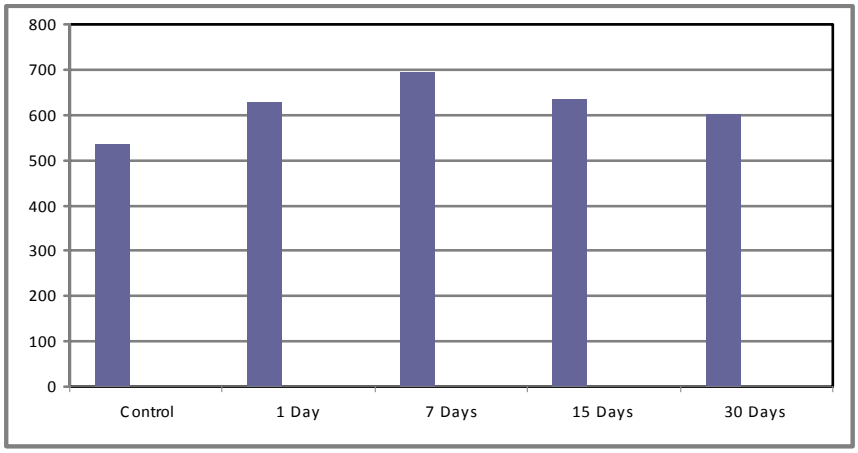


Table (6): Effect of EMF on blood platelets count (10^3/mm ^{3}) of adult male rats blood platelets count (10^3/mm^3)

GROUP	White blood cells (WBCs) 10^3/mm ^{3} (M±SE)
Control	11.35 ± 0. 13
One day	13.65 ± 0. 27*
7 days	15.68 ± 1. 27**
15 days	14. 89 ± 0. 76**
30 days	12. 23 ± 0. 39

All data are Mean value± Standard error N= 6 animals of each group.
* Significant at ($p < 0.05$). ** Highly significant at ($p < 0.01$).



Discussion

The present experimental study showed that the chronic exposure to SMF induced different splenic histological and haematological disruption in albino rats. The earliest haematological responses reported one day after the end of exposure to SMF revealed a significant decrease in haemoglobin, serum iron and hematocrit values. However, no significant change in RBCs was detected.

This decrease could be attributed to the interaction between heme (iron) and SMF where the magnetic field penetrates the body and acts on ions in all organs, altering the cell membrane potential and distribution of ions **(Kula, 1996; Berg, 1993).** Regular exposure to SMF leads to an increase of plasma volume. Therefore, haemoglobin concentration was slightly below normal values in the presence of low serum iron levels **(Amara et al., 2006 ; Chater et al., 2006).** In addition, the static magnetic field may cause cardiovascular stress accompanied with a slow development of mild cardiac decompensation during the exposure period, hence developing heart failure with subsequent passive congestion and stagnant hypoxia **(Walter & Israel 1987; Snover 1989 ; Grawford 1994).** Thus, it can be concluded that as the body adapts to the higher oxygen needs, more fluid would be in the blood. In turn, measured haemoglobin in such cases would be apparently low. This is because it was diluted out by a larger plasma volume. Moreover, the red blood cells looked normal on the blood smear although haemoglobin, serum iron and haematocrit values were significantly decreased, hence resulted in anemia. Usually, this type of anemia is mild and appears like the pseudo-anemia caused by sports. In this respect, regular physical activity, especially extensive running and exercises increase iron loss causing mild iron deficiency **(Bärtsch et al., 1998)**. True iron deficiency can even occur especially when nutritional iron intake is insufficient and iron demand is increased.

From the histopathological perspectives, the spleen which showed congestion or storage of RBCs in the splenic sinusoids was accompanied with a conspicuous thickening in the splenic capsule and trabeculae. In this context **Cesta (2006)** described that the splenic capsule is composed of dense fibrous tissue, elastic fibres, and smooth muscles.

However, animals that depend on running for their survival tend to have rather muscular splenic capsules; they can store erythrocytes and release them into the general circulation when needed for extra oxygen carrying capacity **(Bacha & Linda, 2000).**

The capsule and trabeculae of dogs, horses and cats contained more smooth muscle than that of mice and rats. So, the spleen of rodents do not contracts as rapidly and tends to be varying in their gross appearance **(Valli et al., 2002).**

Thus, the thickness of the capsule, trabeculae and concentration of smooth muscles are very important agents to make strong contraction when the body needs the blood and the smooth muscle concentration may play a role in the immune reactions. Indeed, the thickened splenic capsule and trabeculae after chronic exposure to SMF allow the spleen to contract and eject stored extra erythrocytes from the splenic sinusoids when needed for extra oxygen. This is due to the resultant stagnant hypoxia like status. This view supports the opinion of **Bacha and Linda (2000),** who reported that animals evolved to live at low altitudes often have oxygen loading curves that depend on a high partial pressure of atmospheric oxygen. When moved to high elevations, they often generate extra erythrocytes to compensate for the thin air. These extra erythrocytes are stored in the spleen. Then they would be released when needed to deal with exertion**.** This was also supported by the work of **Pinkus et al. (1986)** in their study on human spleen.

A significant increase in white blood cells and blood platelets count that were accompanied by an enlargement of the white pulp masses was detected one day after the end of exposure to SMF.

It is clear that a splenic white pulp represents an active site of lymphocytic cell proliferation. This is supported by the most recent studies by **Kaszuba et al.(2008)** and **Mohammadnejad et al. (2010),**who reported that actively lymphocytic proliferaton are more sensitive to environmental factors including magnetic fields.

The increase in haemoglobin and haematocrit seven days after sub-acute exposure to SMF may be explained by the installation of hypoxia-like status which is probably resulting from the oxygen binding impairment of haemoglobin or iron metabolism disruption. Thus, exposure to SMF decreased the serum iron level. This is in accordance with previous studies showing that exposure to electromagnetic field induced a decrease in blood iron **(Stashkov & Gorokhov, 1998; Nourmohammadi et al., 2001).** Similarly**, Hachulla(2000)** reported that iron was decreased in plasma of French population living near riverside high-voltage transmission lines.

It is well documented that transferrin controlled transit of iron since intestinal enterocytes increase medullar erythroblasts and allowed recovery of iron after destruction of erythrocytes by macrophagic system **(Wagner, 2000*).***

The increase of haemoglobin and red blood, seven days after exposure to SMF may be explained by the hypoxia-like status. However, the precise way

in which SMF induced hypoxia-like status has not yet been fully clarified. The hypothesis of an action of SMF on the geometrical conformation of haemoglobin was reinforced by the fact that SMF induced a prominent effect on the haemoglobin structure as previously demonstrated by **Amara et al. (2006).**

Recent studies by **Hassan &Abdelkawi (2010)** showed that exposure of the animals to moderate and strong static magnetic fields induced change in the absorption spectra and conductivity measurements of hemoglobin molecules. Furthermore, they found different degrees of globin unfolding. They regarded them as a sign of molecular destabilization. This reflects the function of haemoglobin which would be converted from oxy-haemoglobin to non functional met haemoglobin with decreasing oxygen affinity.

After seven days following the end of exposure to SMF, the histopathology of splenic tissues revealed numerous dilated sinusoids containing some of erythrocytes and/or haemolysed blood. **Faine et al. (1999)** reported large zones of haemorrhage and some features of vascular congestion in the red pulps of infected spleens. In such cases, damage of the vascular walls may be a reason for altering the permeability of sinusoidal capillaries, allowing the leakage of red blood cells, their progressing to haemorrhagic areas and scattering throughout the red pulp. Thus congestion is very likely resulted from disruption of splenic vasculature.

In addition, the white pulps were numerous, fragmented and appeared diffused in an irregular manner throughout the red pulp, such pathologic consequences were confirmed haematologicaly by the significant increase of the number of white blood cells. Thus, it is clear that magnetic field affects the population of lymphatic cells and it has a suppressive effect on immune system.

These findings are in accordance with those of **Attia &Yehia (2002)** who reported a progressive depletion of splenocytes in the white pulp areas in addition to the fragmentation of the tissues. Numerous inflammatory cells, macrophages and megakaryocytes were also noted in the splenic parenchyma and sinusoids, seven and fifteen days after the end of exposure to SMF.

Regarding the increase in the number of macrophages, demonstrated in this study, it was clear that they are defensive and resistant cells. There were several reports showing that under stimulatory conditions and tissue damage, macrophages become more active **(Zidek et al., 1998; Cui & Benowitz 2009)** and their number increase **(Lissbrant el al., 2000).** On the other hand **Simko et al. (2001)** have shown that magnetic field results in a significant increase of the phagocytic activity of macrophages.

Brown haemosiderin granules were seen in the cytoplasm of macrophages after thirty days following the end of exposure to SMF. These granules represent the result of haemolysis of red blood cells.

Haemosiderin was found in all cases that showed large zones of haemorrhage which resulted from disruption of splenic vasculature. Haemolysis of red blood cells results in the release of haemoglobin, which is then phagocytosed by macrophages and stored in the cytoplasm in the form of hemosiderin **(Damjanov, 1996; Faine et al., 1999).** Moreover, the ultrastructural appearance of haemosiderin pigments within sidrosomes in splenic macrophages suggests that erythrocytes degeneration is contributed to be pigment production **(Ward & Reznik-Schüller, 1980).**

A significant decrease in most hematological parameters was reported after fifteen days following the end of exposure to SMF. Our data demonstrated that SMF exposure was associated with significant low levels of RBCs numbers and the haemoglobin was associated with a significant increase in serum iron level. These findings were further confirmed histopathologically in the spleen which showed splenic sinusoids with hemorrhagic or/and hemolysed blood and an increase of hemosiderin granules.

Based on Perl's Prussian blue reaction of iron, the haemosiderin granules observed in the dilated splenic sinusoids which were filled with haemolysed red blood cells. The accumulation of haemosiderin pigments in the spleen as iron storage organ is mainly due to the rapid and continuous destruction of erythrocytes with erythrophagia and breakdown of haemoglobin and its conversation to haemosiderin.

Histopathological features of the white pulps that revealed lymphoid depletion and others which were hyperplastic with proliferation of megakaryocytes. These features observed in this context were confirmed haematologically with significant increase in the blood platelets and WBCs after 15 days following the end of exposure to SMF.

Henrykowska et al. (2009) indicated that exposure to magnetic field induced oxidative stress and free radicals generation in human blood platelets, producing a number of adverse effects and thus may lead to systemic disturbances in the human body.

Thirty days following magnetic exposure, the splenic tissues appeared almost normal and manifested a tendency towards recovery.

Conclusion,

Several experiments are still necessary to elucidate which frequency, intensity, exposure time and other parameters involved with SMF in order to be safe, especially, these concurrent with the environmental pollutants. In turn, we should protect ourselves against this pollutant.

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ETFRC: Enhanced TFRC for Media Traffic over Internet

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Abstract: The evident increase in media traffic over Internet is expected to worsen its congestion state. TCP-friendly rate control protocol TFRC is one of the most promising congestion control techniques developed so far. TFRC has been thoroughly tested in terms of being TCP-friendly, responsive, and fair. Yet, its impact on the visual quality and the peak signal-to- noise ratio PSNR of the media traffic traversing Internet is still questionable. In this paper we aimed to point out the enhancements required for TFRC that enables producing the maximum PSNR value for Internet media traffic. Firstly, we suspected the default value of n that represents the number of loss intervals used in calculating the loss event rate in the TFRC equation. This value is recommended to be set to 8 according to the latest RFC of TFRC. We investigated the effect of modifying the TFRC mechanism on the resulting PSNR of the transmitted video over Internet using TFRC via switching n across the values from 2 to 16. We investigated the effect of such variation over a simulated network environment to study its effect on the resulting PSNR for a number of arbitrary video sequences. Our simulations results showed that running TFRC with n=11 led to reaching the maximum PSNR values among all the examined values of n including its default value. Secondly, we tested the impact on the PSNR of another modification in the TFRC mechanism via switching both values of n and Nfb which is frequency of feedback messages sent by TFRC receiver to its sender every round-trip time RTT. The default value of Nfb is 1; hence we scanned every possible combination of n and Nfb ranging from 2 to 16, and from 1 to 4, respectively and recorded the produced PSNR. It was obvious that several other combinations of n and Nfb produced higher PSNR values other than their default values in the request for comment RFC of TFRC. We hereby suggest using an enhanced TFRC that we abbreviated as ETFRC which has the values of n and Nfb value set to 4 and 11 respectively as a replacement for the traditional TFRC to enable reaching higher PSNR for media traffic over Internet.

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1. Introduction

The amount of media traffic traversing Internet has remarkably increased over the last decade. Applications pushing media traffic such as video on demand VoD, video conferencing, and various video streaming websites have been lately invading the cyber space. The best-effort existing IP infrastructure was not primarily designed to suffice the quality of service QoS requirements of such traffic. Both of the current UDP and TCP have drawbacks when used as the transport protocol for media traffic. TCP seems to break the delay constraints due to its acknowledgments; meanwhile UDP shows aggressiveness in acquiring the available bandwidth to accomplish the streaming task. UDP leaves an unfair share for the co-existing TCP flows which leads to congestion status.

During the periods of congestion; routers tend to discard legitimate packets traversing a certain bottleneck to be able to serve the aggressive media packets. Efforts have been made by researchers to control congestion; they tried to balance between allowing for QoS achievement and acting in a TCP-friendly manner at the same time.

This was via building congestion control protocols that leave a fair share of bandwidth for the concurrent TCP flows traversing across the same bottleneck. Protocols designed for this purpose have been tested regarding compatibility with the TCP-friendliness concept defined in [1].

TFRC presented in [2] was one of the congestion control protocols that managed to achieve remarkable smoothness in the variations of its rate of transmission in addition to fulfilling the TCP-friendliness conditions. For this reason TFRC was the best current promising candidate for media streaming applications, where its smoothness helps in reducing the undesired jitter of the perceived video. TFRC has been extensively tested in terms of fairness, aggressiveness, and responsiveness as in [3] and in

terms of user-perceived media quality on analytical basis in [4]. Testing the visual quality of the media traffic running over TFRC in terms of its produced PSNR was made in our previous work in [5]

This paper aims to reach an enhanced version of TFRC named as ETFRC that manages to produce higher PSNR values than for media traffic over Internet. To achieve this goal a network simulation topology was built to stream a variety of arbitrary video sequences over TFRC. The n parameter that represents the number of loss intervals samples used in calculating the loss event rate in the TFRC equation was switched among different values aiming to determine its optimum value for the best visual quality of video sequences transmitted over TFRC. This quality is measured in terms of the produced RSNR values for the streamed videos. The optimal value for n was found to be "eleven" where the maximum PSNR values were observed. Another investigation was made through switching the values of both of n along with Nfb which is the frequency of feedback messages per RTT. We aimed to figure out the combination of n and Nfb that leads to producing the maximum PSNR values for the video sequences traversing Internet using TFRC. Hence, TFRC is suggested to enhance its mechanism to be ETFRC that uses n=11 and Nfb=4 instead of their default values used in traditional TFRC.

The rest of this paper is organized as follows: Section II covers the TFRC literature and its modified versions tackling the media streaming task over the last decade. Section III explains the enhancement we proposed to TFRC mechanism to produce the proposed ETFRC for media traffic over Internet. Section IV explains our simulation environment, the tool-set used in simulations, the topology of the simulated network, the link parameters set, and the characteristics of the running video sequences. Section IV presents the simulations output focusing on the PSNR values for the tested TFRC in order to show that the enhancement introduced to reach our proposed ETFRC managed to produce the maximum PSNR values. Finally Section V concludes this work.

2. Material and Methods

A. TCP-friendly Congestion Control

TCP friendly congestion control schemes lies into two main categories according to [1]: (i) single rate schemes and (ii) multi-rate schemes. Unicast applications tend to utilize the single rate protocols where all recipients receive data with the same rate. This feature limits the scalability of the protocol towards bandwidth variations that exist in the path to some recipients. Multi-rate protocols are more flexible and enable the allocation of different rates for different recipients which makes it more appropriate for the multicast applications.

Each of those two top categories can be sub-divided into other two sub-categories as follows: (i) rate-based schemes and (ii) window-based schemes.

Some of the rate-based schemes apply the additive increase multiplicative decrease AIMD approach embedded in TCP. Other rate-based just tune their sending rate in accordance with a TCP model. In both cases the reliability feature of TCP is absent. Example protocols that lie in this category are RAP [6], LDA+ [7], and TFRC.

TCP itself is a window-based protocol, yet some problems should be considered when applying this mechanism on multicast connections. Multicast TCP MTCP [8] is an example protocol of this multicast category that managed to deal with these problems.

B. TFRC Protocol

TFRC is the evolution of TFRCP [9]. It was mainly developed for unicast communications but it can be adapted for multicast. Its sending rate is tuned according to the TCP complex equation (1).

$$T = min\left(\frac{W_m S}{RTT}, \frac{S}{RTT\sqrt{\frac{2bP}{3}} + RTO\,min\left(1,3\sqrt{\frac{3bP}{8}}\right)P(1+32P^2)}\right) \quad (1)$$

Where the parameters are as follows:
T: TCP throughput
RTT: Round-trip time
RTO: Retransmission time-out value
S: Segment size
P: Rate of packet loss
b: Number of packets acknowledged by each ACK
W_m: Maximum congestion window size cwnd.

TFRC uses its sophisticated mechanisms to gather the equation parameters. The average loss interval is the chosen method to fulfill the requirements of the loss rate estimation. The loss rate is measured utilizing the latest 8 loss intervals through tracking the number of packets between consecutive loss events. The default number of loss intervals n utilized is recommended to be set to 8 according to the TFRC latest RFC which has the number 5348. The average of a specified number of loss intervals is calculated using decaying weights so that old loss intervals contribution in this average is less. The loss rate is considered as the inverse of the average loss interval size.

Some additional mechanisms are adopted to prevent TFRC from responding aggressively to single loss events, and to guarantee that the sending rate adapts quickly to the long intervals that are loss-free.

RTT is measured by sending feedback time stamps to sender.

TFRC goes through a slow-start phase directly after starting just like TCP in order to increase its rate to reach a fair share of bandwidth. The slow-start phase is ended by reporting a loss event. TFRC receiver updates the equation parameters and feeds them back to sender to adjust its rate every round-trip time RTT. Hence a feedback message is sent once per RTT which means that the default Nfb=1, leading to the recalculation of the sending rate for TFRC only once per RTT.

TFRC adopts additional delay-based congestion avoidance by adjusting the inter-packet gap which would be applied in some environments that do not support the TCP complex equation.

TFRC main advantage is its stable and smooth sending rate variations. This feature fits in the media application transmission besides being responsive to the co-existing traffic in a TCP-friendly manner.

C. TFRC Enhancement Attempts

Several attempts were made to enhance the performance of TFRC in order to suit the media traffic requirements such as what discussed in the following lines:

In [10] authors observed some performance degradation for TFRC over wireless networks, and hence they tried to customize it through a more advanced equation. This equation was reached via modeling wireless TCP rather than wired TCP. Applying this equation led to a remarkable throughput increase with about 30% over wireless networks with loss of 10% while maintaining the TFRC main features of TCP-friendliness and smoothness.

In [11] an attempt was made to enhance TFRC performance over mobile and pervasive networks that focused on overcoming data losses due to the frequent loss of connectivity. The method used was applying a mechanism that resembles Freeze–TCP when a disconnection incident is expected. Additionally, a probing mechanism to enable speedy adaptation to new network conditions was proposed.

Another enhancement was made in [12] where authors tackled the problem of keeping fairness and smoothness of TFRC media streams when existing among other streams. They proposed MulTFRC that was successful in keeping low delay values to satisfy the media QoS requirements.

In [13] the enhancement made was that authors computed the rate gap between the ideal TCP throughput and the smoothed TFRC throughput replacing it. Any rate gain from this gap was opportunistically exploited via video encoding. A frame complexity measure is specified to determine the additional rate to be used from this rate gap, and then the target rate for the encoder and the final sending rate are negotiated through the same frame of complexity.

In [14] authors suggested incorporating the selective retransmission concept into TFRC. Retransmission of lost packets is done selectively when no congestion case is present. Selective retransmission was shown to have a significant positive effect on the streamed media quality over TFRC.

In [15] authors claimed that TFRC does not perform satisfactorily on multi-hop ad-hoc wireless networks. They saw that TFRC sending rate can be deceived by MAC layer contention effects such as retransmission and exponential back-off. Hence, they proposed enhancing TFRC by introducing RE-TFRC. RE-TFRC used measurements of the current round-trip time and a model of wireless delay to prevent TFRC from overloading the MAC layer while keeping its TCP-friendliness feature.

In [16] a performance analysis of a QoS-aware congestion control mechanism named guaranteed TFRC (gTFRC) was presented. gTFRC was embedded into the enhancement transport protocol (ETP) that enables protocol mechanisms to be dynamically controlled. gTFRC managed to reach a minimum guaranteed transfer rate for any given round-trip values and any network provisioning conditions as well

In [17] an extension for TFRC was suggested in order to support variable packet size streams. Variable packet size is already utilized over Internet in both video and voice over IP VoIP transmission. This enhancement of TFRC was made through a modified concept of TCP-friendliness and was validated to perform better than the original TFRC with the packets of variable sizes.

In [18] authors proposed an enhanced TFRC based on step explicit congestion notification ECN making. They found that TFRC has poor performance over wireless networks because it accounts for wireless losses as congestion. They managed to reach higher throughput via utilizing the appropriate loss differentiation and congestion notification. Their proposed enhanced TFRC kept reasonable friendliness to the co-existing TCP flows according to their simulation results.

The above enhancement attempts focused on achieving a better quality of data transmitted over TFRC while maintaining its main advantageous characteristics of being TCP- friendly and fair in acquiring a bandwidth share.

3. Enhanced TFRC ETFRC Protocol

The enhancement that we introduced to TFRC to produce ETFRC was increasing the number of sample loss intervals n used to calculate the rate of packet loss P from n=8 to n=11 We also studied the effect of combining the different values of n along with other values of feedback frequency represented by Nfb parameter. The effect of varying the Nfb solely was studied in [19]. Our goal is to demonstrate through simulations that combining the values of n=11 and Nfb=4 leads to the maximum PSNR values for the transmitted video over TFRC. For the sake of completeness, other effects of such increase on the TFRC mechanism of work should be discussed. In [20] authors discussed the impact of increasing the feedback frequency from different perspectives that we summarize here.

A. The Impact of Increasing Nfb on TFRC Mechanism

To have a better view for the background image of TFRC, we have to understand that there are two key parameters that drive the TFRC mechanism in Eq.1 which are the loss rate p and the experienced RTT. In our work we used the value of Nfb=1 as a reference value as this frequency is considered as the default of TFRC. Simulations made in [20] showed that feedback frequencies greater than one per RTT lead to lower drop rate. It was also clear that the feedback frequency is not correlated to P, where both values were randomly distributed. Hence, Nfb has no critical effect on P from the macroscopic perspective.

The analysis made in [20] for TFRC demonstrated the impact of increasing the feedback frequency on RTT, it showed that during the slow-start of TFRC the link suffers from under-utilization as well as slow convergence. For the first hundred seconds the value of RTT in TFRC equals to that of the link propagation delay, meanwhile after this period, TFRC enters the steady state where the increase in feedback frequencies causes the experienced RTT to increase.

B. The Impact of Increasing Nfb on Sending Rate

Researches also noted that when the feedback frequency increases the rate of the senders tend to decrease, which seems to contradict with what was expected of decrease in network congestion and the decrease in RTT consequently. The higher feedback frequency was found to improve the accuracy of the estimated RTT and the accuracy of the estimated p in consequent. This enables TFRC to acquire the network resources with the minimum required sending rate due to the fact that the more accurate the sense of the network congestion parameter the faster the adaptation of TFRC to the network conditions.

C. The Impact of Increasing Nfb on Network Parameters

Increasing the feedback frequency of TFRC from one to four had a positive impact on its responsiveness. However the resulting fairness depended on how lost packets were distributed among flows. The link utilization also followed the same behavior of increase like that of responsiveness when having multiple feedbacks per RTT.

4. Simulations Environment

This section describes our simulation environment used to perform the PSNR evaluation of the media traffic over TFRC. The main three components of these simulations are the tool-set used, the topology of the simulated network created using ns-2.30 [21], and the group of video sequences arbitrarily chosen for this purpose.

A. EvalVid Tool-set and Evalvid-RA

In [22] Chih-heng Ke et al. proposed a novel and complete tool-set for evaluating the quality of MPEG video delivery over simulated networks environment. This tool-set is based on the EvalVid framework [23]. They managed to let ns-2 as a general network simulator replace the EvalVid simple error simulation model through extending its connecting interfaces. This allowed researchers and practitioners in general to simulate and analyze the performance of real video streams with consideration for the video semantics under a vast range of network scenarios.

The tool-set valuable feature is that it allows for the examination of the relationship between two well-known objective metrics for QoS assessment of video quality of delivery which are the PSNR and the fraction of decodable frames.

As shown in Fig. 1 the concept of this tool-set is built upon creating trace files from an encoded raw video. These files are text files and are fed into the simulation environment to be used as traffic generators based on the encoded video parameters. The output of the simulation process can be decoded as well to produce the output video file of the simulation. The quality of the output file and the original video file can then be compared to obtain a representative PSNR value for the media quality of the produced video from simulation.

EvalVid-RA proposed in [24] is an extension of EvalVid tool-set that supports the rate adaptive media content as well as the TFRC protocol in the simulation environment, thus it was chosen to be used in this work.

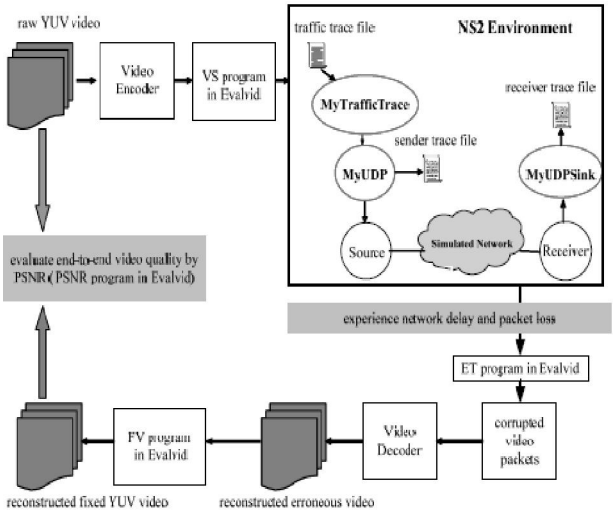

Fig. 1. EvalVid Tool-set

B. Simulation Network Topology

Our simulation topology in this paper is the same simple topology used in [24].

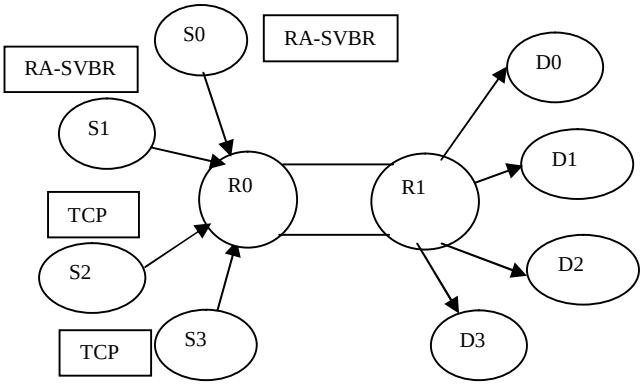

Fig. 2. Simulation Topology

As shown in Fig. 2, it is a simple dumbbell topology composed of four traffic sources and four traffic destinations. We used ns-2.30 as our network simulator to build this topology.

Both S0 and S1 are fed with the simulated video trace files while S2 and S3 generate TCP traffic. The video traffic is shaped variable bit-rate traffic rate adaptive (RA-SVBR), and the bottleneck between the routers R0 and R1 is 40Mbit/s link with propagation delay of 10ms. The access network capacities were set to 32Mbit/s with 5ms delay producing a one-way propagation delay of 20ms. The fair share after starting all sources was over 625Kbit/s and both R0 and R1 routers used ordinary random early detection (RED).

Using ns-2.30 allowed for testing TFRC with different Nfb values aiming at reaching its optimum value required for ETFRC which enables for the best PSNR values.

C. The Video Sequences Used in Simulations

Two video sequences were used in our simulations as shown in Table 1. They are provided for research purposes by Arizona State University research group in [25].

$$PSNR\ (dB) = 20\ \log_{10}\frac{V_{peak}}{MSE} \qquad (2)$$

$$V_{peak} = 2^k - 1$$

Where k and MSE are as follows:

k: the number of bits per pixel

MSE: the mean square error of the luminance component.

They were used in YUV format where they are firstly encoded using ffmpeg.exe and then passed by the mp4.exe which are both component files of the used tool-set.

A brief description for each of the videos content is presented as follows:

Bridge-close: a close scene of Charles Bridge

Mobile: panning of moving toys

The final output of the tool-set is a text file that contains a table of two columns where the PSNR value of each compared video frame calculated according to equation (2) .is recorded in decibels (dB) corresponding to their frame numbers. We utilized the mean PSNR value for each simulated video file for our evaluation purpose to be compared with the reference mean PSNR produced by TFRC having the default Nfb value of one.

The following table shows the video sequences used and their number of frames and complexity of motion:

#	Video	# of Frames	Motion Complexity
1	Bridge-close	2001	Low
2	Mobile	300	Medium

The resulting text files are then fed into the ns-2 simulation file where the output is concatenated through et_ra.exe tool and then decoded. The decoded file quality is the compared to the original file quality using the psnr.exe program as demonstrated in Fig. 1.

5. Simulations Results

The goal of building and running the simulated environment explained above was to investigate the effect of switching the n and Nfb parameter over a range of values on the resulting PSNR values of the output files.

Our results are based on calculating the mean PSNR values for each video sequence which are the results of comparing the output video files of simulation over TFRC protocol and the original video files. Those PSNR values were calculated using the psnr.exe program as a part of the EvalVid tool-set. The program compares each output video file to the original file on frame-by-frame basis. It produces a text file that contains a PSNR value for each of the video frames. The mean of those PSNR values were computed to be compared to those of the default PSNR values of the TFRC

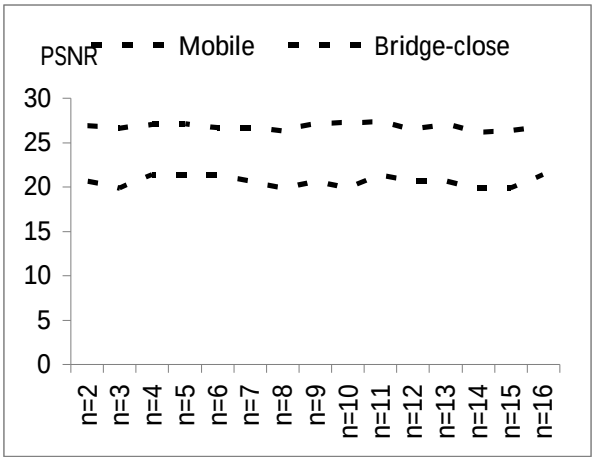

Fig. 3 PSNR Values Vs. *n*

Knowing that the default value of n in TFRC is "8" and of Nfb in TFRC is "1", we managed to switch n over a range of values from 2 to 16 and Nfb over the values of 1, 2, 3, and 4.

Our first finding was that the PSNR values of the output files all lie in the acceptable range at the chosen values of n and Nfb. This means that TFRC is a suitable candidate for running the media traffic from both points of views of TCP-friendliness and media quality maintenance. The output files were visually meaningful. They had a degraded quality with respect to the original video files, but all the files were considered to be visually acceptable by human eye as well as PSNR values.

Our next goal in this paper was specifying the exact combination of values of both n and Nfb that produces the maximum PSNR for the output video files, and that would be set as the defaults in the suggested ETFRC. To achieve this goal we recorded the PSNR for each of the values chosen for n and Nfb. Fig. 3 represents the PSNR values of both simulated videos versus the range of values scanned for n while Fig. 4 represents the PSNR values of one of the videos versus all the possible combinations of n and Nfb over their scanned ranges for testing.

We can also note in Fig. 3 that n=11 leads to the maximum PSNR values among the other tested values. While in Fig. 4 we can notice that the combination of n=11 and Nfb=4 is the optimum for having maximum PSNR for the tested video.

We believe that our simulation results are helpful for measuring the performance of the TFRC from a point of view that has not been thoroughly tackled before which is the PSNR of videos running over it.

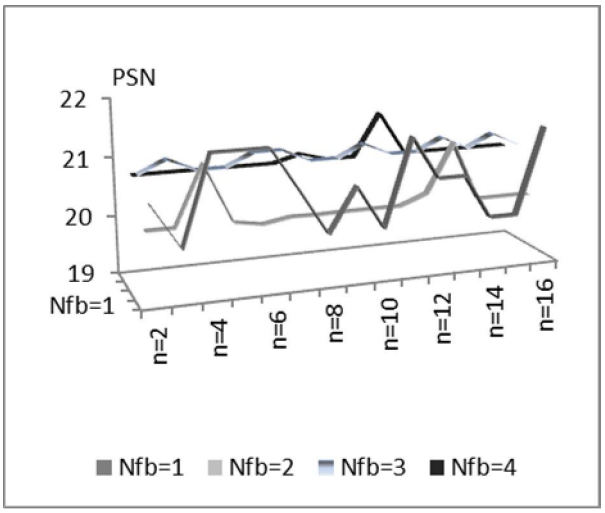

Fig. 4 PSNR Values Vs. n and Nfb

6. Conclusion

The problem of Internet congestion control has been handled by researchers over the last decade. It was observed that the quantity of media traffic traversing the Internet has tremendously increased due to the increase in the number of emerging applications running such traffic which led to worsening the case of congestion.

Several congestion control protocols have been developed to face the problem and also have been tested from the TCP-friendliness point of view and achieved promising performance in many cases, but the media traffic currently booming over Internet imposed some additional criteria on the congestion control protocols other than just being TCP-friendly and fair in bandwidth acquiring. These criteria focused on delivering the media traffic with an acceptable PSNR quality leading to a visually meaningful and acceptable traffic.

TFRC according to researches is a good candidate protocol for this target. It can balance between accomplishing the TCP-friendliness task and allowing for some QoS constraints to be met. Several researches pointed out that TFRC with its current mechanism of work is not the ideal protocol for congestion handling, and hence many enhancement attempts were made to reach a more suitable form of

it. A number of research work targeted the evaluation of TFRC in terms of TCP-friendliness and fairness and many tests were done for this purpose either in the simulated environments or in the real-world. TFRC has also been tested so far regarding the quality of the media traffic running over it.

This work managed to find an enhanced version of TFRC named as ETFRC through changing the default values of both parameters n and Nfb in TFRC from eight to eleven and from one four respectively to form the ETFRC. This was by testing the quality of a group of videos when transmitted over TFRC while switching the values of n and Nfb over a range of nominated integers. This testing utilized the simulation environment and was made in terms of the visual usefulness of the received video files. It was also made in terms of the mean PSNR values in dB of the output files of the simulation process when compared to the originally transmitted files over TFRC.

TFRC was shown to produce acceptable quality for the received video files. This emphasizes the fact that TFRC is still the candidate for the congestion control problem solving. It was also shown through the simulations results that the performance of TFRC in terms of quality was enhanced slightly with the above suggested changing in defaults

We hereby propose the ETFRC protocol as an enhancement for TFRC where the number of sample loss intervals used is eleven instead of eight and the feedback frequency utilized value is four instead of one. We also believe that this work can be helpful for researchers handling the congestion control problem and researchers trying to enhance the performance of TFRC in order to increase its capabilities of being the congestion control problem solution.

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Combination toxicity effects of heavy metals on terrestrial animal (Earthworm- *Eisenia andre)*

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ABSTRACT: Combination toxicity of heavy metals to earthworms has been studied for the first time. Three metals, namely Cadmium (Cd), Lead (Pb) and Zinc (Zn) were used as toxicants. The study was carried out in line with the OECD recommended procedure for ecotoxicology tests of chemical substances to soil. Hence, the OECD approved earthworm species Eisenia Andrei was used as biological test organism. OECD artificial soil was used as test substrate. The parameters of interest in the study were the effect of logarithmically increasing concentrations of single, and finally of combinations of metals, to reproduction and body growth of earthworms. These parameters were chosen for the study since sublethal effect on growth and reproduction are of more ecological relevance than an acute toxicity effect studying mortality. Growth was measured as the increased in body weight of the worms in time. Reproduction was estimated based on the number of cocoons produced per worm and the number of emerged juveniles produced per cocoon. The EC50 values of the effect of the toxicants to the organisms were estimated by an extended logistic model which is able to take into account the presence or absence of hormesis in calculating EC50 values. The EC50 values after three weeks of the three metals to growth and cocoon production were first determined singly. Cocoon production and juvenile numbers appeared to be more sensitive parameters than growth. A continuous increase in weight was observed in almost all tested concentrations of each metal except at the highest concentration of lead. Cd appeared to have the strongest adverse on cocoon production, followed by Zn, and then Pb. This is judged from the observed EC50 values of each of the metals on cocoon production. The observed EC50 values were 104.0mg/kg (1.076 mmol/kg), 418.3mg/kg (6.398 mmol/kg), 1570.3mg/kg (7.578 mmol/kg) for Cd, Zn and Pb respectively. Based on the observed EC50 values of the single toxicity tests, combination toxicity tests were carried out for Zn & Cd, Cd & Pb and Pb & Zn. Zn & Cd were found to be antagonistic (less than concentration additive) other tests where not interpretable. The ecological implication of the result of combination toxicity test of Cd & Zn is that single toxicity test alone may not indicate the fate of earthworms exposed to sites polluted with combinations of metals.

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Keywords: toxicity; heavy metal; terrestrial animal; Earthworm; *Eisenia andre*

INTRODUCTION

A literature inventory using a computer mainframe programme Biosis Data base has revealed that combination toxicity of metals has never been studied using earthworms or other terrestrial organisms, apart from bacteria. It is only in aquatic literature that such studies have been recorded. Pharmacological achievements and other cases of successful development of chemical mixtures for economic reasons, have been reported to originate from ancient knowledge of the advantages of mixtures of chemicals over chemicals applied singly (Marking, 1977). Some chemicals are reducing or increasing the toxic effects of the other toxicants for target organisms (Marking, 1977).

Environmental risk assessment of metals to the soil compartment has always been tested with single toxicants in the laboratory by using earthworms or other species as biological test organisms. Yet in nature earthworms, like other terrestrial organisms in contaminated ecosystems, are usually exposed to mixtures of toxicants. This study, which is the first of its kind, is aimed at determining the toxic effects of combinations of metals to earthworms of the species Eisenia andrei. The study should prove the accuracy of single toxicity tests in determining the toxic effect of metals to earthworms. Comparisons of the result of these experiments can be made with available findings will help ascertain whether or not combination toxicity of metals describes the toxicity metals to earthworms in a better way than when metals are tested singly. This will, of course throw more light on the approach of toxicity tests of metals to estimate risks for the polluted soil compartment.

The importance of earthworms has been long recognized. Darwin, as far back as 1881 has this to say about earthworms and 1 quote from Lee (1985): "It may be doubted whether there are many other animals which have played so important a part in the history of the world, as have these lowly organized creatures". Earthworms have been used as human food and to alleviate some illnesses, nut there scientific relevance originates from the fact that they have been known to be important soil fauna (Edwards & Lofty, 1977). They enhance soil fertility in many ways, for example by mixing of the soil, formation of water stable aggregates,

aeration of the soil and improvement of its water holding capacity by their burrowing activities (Edwards & Lofty 1977). Therefore substances that effect the ecology of earthworms will also interfere with the productivity of the soil. Earthworms have specific metal binding proteins (Satchell, 1983). They have been reported by many authors to be able to take up and accumulate heavy metals in their tissues. These metals could be accumulated further in birds and animals that feed on earthworms and finally in man. This type of problem is expected to occur more in industrialized countries (Lee, 1985). Earthworms exist in large number in many soils. Some species can be easily grown in the laboratory. They can be readily collected and identified (Van Gestel, 1991). Thus they are cheap test organisms. It is because these profitable characteristics of earthworms that they have been selected as indicator organisms for testing ecological effects of industrial chemicals (OECD, 1984; EEC, 1985).

The toxicity of metals is concerned with some 80 elements and their compounds (Musch, 1992). Cd, Zn and Pb have been selected for this study because they occur commonly together in minerals and soils (Musch, 1992). Cd is usually a by-product of Zn refining, so it is the level of production of zinc and not the demand for Cadmium that determines the supply of Cd (IHE, Ecotoxicology Workshop, 1992). These three metals are among the suspected carcinogenic metals. Zinc and lead have been proven to be carcinogenic to experimental animals (Musch, 1992). Their tested concentrations are within the range they occur at polluted sites. They have been found to occur in concentration of up to 0.988mol/g, 445mol/g and 60.5mol/g respectively for Cadmium, zinc and lead at polluted sites in north – Western Europe (Posthuma, 1992). They have been selected, among other metals, for the validation project operating in Budel, a metal polluted site in the southern part of the Netherlands. The result of this research will provide part of the database for that project.

Soil characteristics can have great influence on the absorption and therefore on the bioavailability of chemicals in soil (Van Gestel, 1991). Therefore it is pertinent that earthworms toxicity test should be carried out with a standard substrate like OECD artificial soil. For reasons of standardization and reproducibility, OECD artificial soil was chosen as test substrate. This artificial soil was made specifically for these tests and has the same absorption capacity as a typical loam soil (Van Gestel, 1991).

A parameter like acute toxicity effect can only give information on the number of organisms lethally affected by the toxicant. Earthworms at polluted sites are exposed to toxicants all through their life time and acute toxicity tests alone cannot give information on what an organism suffered before death. A toxicant which does not lead to immediate death, but affects example the genetic constitution or reproductive capacity or growth of an organism, may disturb the ecological balance of that population. According to Spurgeon et al. (1993), reproduction and growth disturbances are far more likely to mediate population effects, and reproduction is likely to be of particular importance in ecotoxicological risk assessment because of its influence on population dynamics. From this view point, sublethal effects on reproduction (cocoon and juvenile production) and growth were chosen as test parameters.

2. MATERIALS AND METHODS

2.1. TYPES AND SOURCES OF TEST MATERIALS

2.1.1. Biological Material

Earthworm of the species *Eisenia andrei* were used as test organisms. All the worms used were adults of about 18 weeks of age with well developed clitellum. They were retrieved from RIVM earthworm mass culture, reared following a standard operation method (Van Gestel, 1991).

2.1.2. Food

Cow dung was used as food for the worms. The dung was obtained from a local farm, oven-dried over night and ground to a diameter of 0.5

2.1.3. Substrate

OECD artificial soil was used as test substrate. It was prepared in line with the OECD guideline for testing chemicals 207, entitled "Earthworms Acute Toxicity Test", adopted 4th April, 1984, as updated by Van Gestel (1991). The different percentages of substances below used for the artificial soil were weighed into a container (big enough to make 12.50 kg of soil at a time) and mixed together with a cement mixer. The humidity and pH of the soil before being used for tests were aimed to be 55% and a pH 5.0^{+}–0.5, respectively, which was checked in random samples from the test series.

2.1.4. Metals

The metals were added to the artificial soil in the form of nitrate salts; that is cadmium nitrate ($Cd(NO_3)_2.4H_20$, Zine Nitrate ($Zn(NO_3)_2.6H_2O$ and Lead Nitrate ($Pb(NO_3)_2$) respectively.

2.2. PROCEDURE FOR SINGLE AND COMBINATION TOXICITY TEST EXPERIMENTS

The experiments were carried out in two consecutive parts. The first part consisted of the single toxicity effect tests of the individual metals. This part is necessary to provide the necessary data needed for the combination toxicity tests. The second part consisted the combination toxicity effect tests of the different combinations of the metals used for the combination toxicity test. All the worms used for both the single and

combination toxicity test passed through three major phases as shown below namely (Figure 1):

a. The Acclimation Phase
b. Metal Exposure Phase
c. Cocoon Incubation Phase

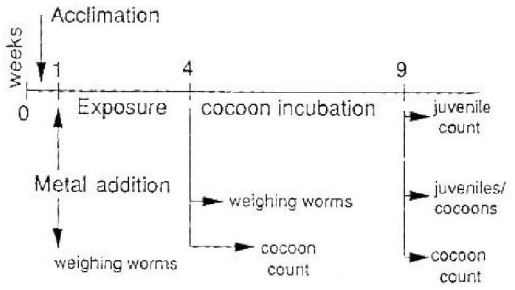

Figure 1. Acclimation Phase

2.2.1. Single Toxicity Tests

Acclimation. The test worms were passed through an acclimation phase in clean artificial soil. This is necessary for the worms to get used to living in a surrogate environment in the laboratory. For this purpose, 24 test jars were labeled for the five different tested concentrations of the metal and a control (each concentration, including the control had 4 replicates).

The moisture contents of the artificial soil to be used was raised to 20%. This was done by adding an equivalent of 20% of the dry weight of the soil in the form of demineralised water. The soil and the water were then mixed together with a kitchen mixer. The 20% humid soil was left to stand for a minimum of 5 days and a minimum of 7 days for a microbial population to develop before being used for the experiment.

At the end of this period, the moisture content of the was raised to 55% by adding the percentage difference of 35% of demineralised waster.

Two different samples of ca. 20g of the soil were then taken pH and humidity measurements respectively. An amount of 625g of 55% humidity soil was added into each jar. Holes were made in the centre of the soul content of the jars and 6.3g of the 55% wet weight of dung was put in the holes. The holes where up by slightly shaking each jar with hand.

Adult worms were hand-picked from the mass culture and put in a big petridish with moistened filter paper. The worms were washed with tap water in small plastic containers in batches of 10 and surface dried by keeping them on humid filter paper for some seconds before weighing. Surface attached water was lost by this process. The worms were weighed in batches of 10, but individual weight was determined for one of the replicates of the 6 concentrations. Each set of 10 worms was put in one of the test jars which already had soil and dung.

Jars were covered with glass Petri-dishes to allow enough air to reach the worms, minimizing evaporation, and preventing the worms from crawling out. The average wet weight of the worms was 0.360+_ g. each set of 10 worms were put in the test jars which already had soil and dung.

The weight of each test jar with soil, worms, dung and lid were taken. The jars were then put in a climate-room (temperature control-room) at a temperature of 20°c+_2 and an illumination of 400-800 lux. Minimum-maximum thermometers were also put in the cabinets were jars were kept, together with humidity chart. This is for constant observation of the temperature and humidity respectively. Appendix 19 shows one of such charts.

This phase lasted for a week. The worms were then ready for the text phase, that is the metal exposure phase after washing, drying and weighing. Samples of the soil were taken to determine if there was change in pH of the soil after the acclimation phase.

Cocoons produced during this acclimation phase were washed from the soil and the numbers of cocoons produced per jar/per worm were noted. This observation was made to assure that all the worms used had reached maturity actually of age of reproduction.

Metal exposure. The different nominal concentrations of metals tested were in Figure 2 (mg/kg):

- Cadmium: 0, 3.2, 10, 100, 320
- Lead: (First experiment): 0, 10, 32, 100, 320, 1000
- Lead: (Second experiment): 0, 50, 158.1, 500, 1581.1, 5000
- Zinc: 0, 10, 32, 100, 320, 1000.

Figure 2. Metal exposure: The different nominal concentrations of metals tested were in (mg/kg)

Two experiments were run for lead. The first one showed only minor effects on cocoon production, i.e. EC50 was not reached. Therefore, the second test was run with higher lead exposure. From here on, only the results for the interpretable experiment for lead (the second) are shown. Original data for both experiments are summarized in the Appendix section (Appendix 2 and 4).

Below is a typical approach for the exposure of worms to metal "X" where "X" could be Cd or Zn or Pb. A number of 24 test jars where labeled (4 replicates per concentration). The moisture content of the artificial soil was raised to 20% with demineralised water and the pH was taken. The soil was left to stand for a minimum of 5 days and a maximum of 7 days for the establishment of a microbial population.

The stock solution of each metal was made from their Nitrate salts. Equal amounts of Nitrate were maintained each tested concentration of the three metals. This was achieved by calculating the concentration at which the highest amount of Nitrate was obtained; this

was at the highest concentration minus the amount already added through the nitrate salt in that particular concentration. The difference was made up with the addition of sodium nitrate. Thus in the control, the highest difference was made up with the addition of sodium nitrate. Thus in the control, the highest amount of sodium nitrate was added while none was added to the highest tested concentration range of the metals.

The exact amount of stock of the metal needed in 1 kg dry soil was pipetted into a 250 ml flask. This was prepared in duplo. Twelve 250 ml flask with 6 different concentrations (in duplicates) of each tested were prepared by adding calculated amount of stocks. Each flask content was then made up to 250 ml fluid liquid content with demineralised water.

The 20% humidity soil was weighed out in batches of 1200g. Each batch was put into a bowl of a kitchen mixer placed under a laminal flow hood.

The content of the 250 ml flask was then added each 1200g weight of 20% humidity soil starting with the control. After emptying, each flask was rinsed with 100 ml of demineralised water and this was added to the soil content making the total moisture content of each initially 20% humidity soil to be 55%. The final weight of the soil sample in the bowl then became 1550 g of 55% moisture content. This 1550g of soil was mixed for about 1 minute with the barters of the mixer. A amounts of 625 g of the 55% humidity soil were weighed out in 2 different jars labeled A and B. the remaining soil from the 1550 g soil was put in another flask for chemical analysis.

The other replicates of the same concentration were also treated as above and 2 different 625 g weight of soil were weighed out and put in jars labeled C and D. a similar procedure was followed to prepare all the different concentrations and the controls. Hence, each tested concentration and the control had four replicates of soil samples in the jar.

All the 24 jars were put in the climate room for 1 week to allow for metal absorption and desorption to and from the soil. This started at the same day at which the acclimation phase of the worms was started. At the end of each week 6.3g wet weight of 55% humidity dung was added to each jar of the polluted soil as at phase A. the phase A jars were emptied in a flat plastic container one after the other, and the worms were hand picked, washed with tap-water, dried on a filter-paper, weighed and put in the exposure jars. Lids were put on the jars. Then the weight of the jars, including worms dung, soil and lids, were taken. The jars were taken then put in the climate room.

The exposure phase lasted for three weeks. Every week, the water content of the jars was determined by taking the weight of the jars. The weight of the content for a particular week minus the weight of the previous week gave an insight into the amount of moisture lost. Moisture loss was corrected by adding an amount of water equivalent to the loss amount of demineralised water every week.

At the end of the third week exposure the temperature of the cabinet pH, and the humidity of a sample of the test jars were taken randomly from different test jars. The worms were retrieved by washing off the soil through a 2 mm sieve. This 2 mm sieve had another 1 mm sieve underneath to collect small sized cocoons that might pass from the former. The number of cocoons produced were counted by eye observation with the aid of tweezers. The weight of each set of worms in the jars were taken after washing and drying.

Cocoon Incubation. The cocoons produced per jar were taken from the sieves, and were put in petridishes containing 65g of 35% wet weight of unpolluted soil mixed up with 1g of dung. The cocoons were again covered up with another 65g of 35% wet weight of soil mixed up with 1g of dung.

The Petri dishes were put in the climate room for 5 weeks for incubation. Each week the moisture loss was made up as in case of the worms. At the end of the 5th week the juveniles from hatched cocoons were calculated after washing out the soul through a 0.5 mm sieve.

2.2.2. Combination Toxicity Tests

At the of the single toxicity test part, the EC50 of the three different metals on growth, cocoon production and juvenile production were estimated statistically. This was done by using a statistical model, written in Genstat 5, which performs curve-fitting to estimate toxicity parameters in dose-effect research. The model is a standard logistic model which is able to take into account the possibility of subtoxic stimulus (hormesis) in calculating the EC50 (Van Ewijk & Hoekstra, 1992).

The toxic unit approach for the combination toxicity test was applied to toxicity data for cocoon production rather than body weight. This is because, the single toxicity tests showed consistent and severe effects of each of the three metals on cocoon production against insignificant effects on growth (see Result section).

The combination studied for their effect on cocoon production were:

Cd & Zn
Cd & Pb
Pb & Zn

The basic data for the combination toxicity test in this study were the EC50 values of the metals tested singly on cocoon production. The EC50 of each metal on cocoon production also refers to 1 TU of the metal based on the definition of 1 TU for a singe. To obtain the theoretical 1 TU for two metals, ½ of the EC50 (1 TU) of the individual metals in the mixture were added together. This theoretical TU was usually made to be at concentration 4. the upper and lower concentration ranges were obtained by dividing or multiplying the concentration of the theoretical 1 TU with 6 root 10, 4

root 10, or square root 10, increments or decrements of 4 root 10 were chosen to have more narrow adjacent concentrations around EC50 expected. Four replicates of jars per concentration were used. For the combination toxicity test of Cd & Pb and Pb & Zn two replicates per concentration were used, since twelve different concentrations were applied rather than six, as usual in single toxicity tests. The same test substances, method of addition of metal and exposure method of the worms as in single toxicity test phase were applied.

2.2.3. Chemical Analysis of Soil Samples

Chemical analysis was carried out to determine the amount of metals that the worms were actually exposed to after absorption and desorption of metals to and from soil. The background concentrations of the metals in the unpolluted soil samples were also determined. For single toxicity tests Cd, Zn and Pb the metal concentration at the beginning and end of the experiments were determined, but since result of the analysis showed no difference between the amount extracted at the beginning and the amount extracted at the end, samples for chemical analysis were only taken at the end of the experiment for Pb and the combination toxicity tests.

Known amounts of soil samples were randomly taken from each replicate of the different tested concentrations of the metal. The samples were extracted with 0.1M $CaCl^2$ and 0.43 N HNO_3 separately, and shaken for 24 hours. The metal ion concentrations in the solutions were measured by Atomic absorption Spectrophotometer (flame-AAS). Measurements of total metal contents through completely dissolving the samples in heated strong acid, and measuring metal ions in solution by AAS were also done. All chemical analysis were downe by the FCT (RIVM – department of physical and Chemical Techniques).

2.2.4. Statistical Analysis

The data were analyzed with a logistic model as described by Van Ewijk and Hoekstra (1993). The logistic model describes a situation without hormesis. An extension of the model is able to incorporate hormesis, and to assess its significance. In the non-hormesis model, the dose-response curve is given by three parameters.

B = Slope parameter, indicating steepness of slope at the EC50

M = Estimate of the in (EC50), from this, EC50 itself can be calculated

C = Estimate of performance when toxicity is absent.

From the estimate of M, the EC50 was calculated. From the accuracy of the fit, the range of the 95% confidence interval of the EC50 estimate was calculated. The hormesis model is determined by an additional parameter, namely:

F = Value to express hormesis, if significant

The models were fitted by least-square estimation, and results are presented for the non-hormetic model if hormesis was not significant ($P>0.05$), or if the hormetic model showed a worse fit than the non-hormetic model. In the latter case, both results will be shown. The accuracy of the fit is given by the models through the percentage of variation accounted for; a high value indicates an accurate fit.

3. RESULT

3.1. ABIOTIC FACTORS

3.1.1. Total and Extractable Amounts of Metals

Nominal, extractable and actual concentrations of metals in the single toxicity test of Cd, Zn, Pb and in the combination toxicity test of Zn & Cd, Pb, Cd and Pb & Zn, respectively, were measured during the experiments. There is no difference between the amounts of metals extracted at the beginning and at the end of each experiment except for Zn, for which a peculiar difference exists between the amount of metals extracted at the beginning and the end. Since 16.4 mg Zn/kg soil was extracted at the beginning, the low concentration recorded at the end of the experiment might be probably attributed to an analytical error. It can be conclude from all measured metal concentrations, that the worms were exposed to the same concentrations throughout the exposure periods. The detailed data are summarized in the Appendix section (see Appendices 6-18).

Nitric acid prepared to have extracted a higher proportion of added metals than calcium chloride based on the percentages estractable amounts of metal by the two substances. Whereas $CaCl_2$ extracted on the average 15% of Cd, 28% of Zn, 0.8% of Pb, HNO_3, extracted 70% of Cd, 69% of Zn and 69% of Pb.

Also, in the combination toxicity experiment with Cd and Zn, $CaCl_2$ extracted 15% of Cd and 19.7% of Zn while HNO_3 extracted 40% of Cd and 60%.

The desired similarly of concentrations of nitrate in all the tested concentrations of the metals was almost completely achieved for Cd, Pb and Cd & Zn, and for Zn up to concentration 4 (for details: see Appendices 6-18). However, there were differences between the amounts of nitrate added among metals, depending on the amounts of nitrate added in the highest concentration. Therefore, the nitrate concentration in all Cadmium exposure groups was lowest, and increasing in the order Pb (first experiment), Zn, Cd & Zn and finally Pb (seconf experiment).

3.1.2. PH and Humidity Readings

Table 1 shows the Ph and humidity measurement of the different OECD soil samples using the experiments. Apart from the combination toxicity tests of Cd & Pb, and Zn & Pb which disrupted due to factors of which lack of optimum Ph is suspected to be one and Cd & Zn combination toxicity test in which the humidity measurement was 37% in all the other tests, the soil pH

and humidity were on the average of 4.76-5.90 and 50.75-57.90% respectively.

Table 1 shows the Ph (at the beginning and end of experiment) and humidity (beginning of experiment) of the OECD soil used in each toxicity test of metal(s).

Table 1. Abiotic factors measured during the exepriments

Added metal	Average of pH Measurement		Average % Humidity
	Begin	End	
Cd	4.82	n.d.	51.80
Pb(1st)exp	4.76	5.40	50.75
Zn	4.76	5.07	57.75
Pb(2nd)exp	5.30	5.60	55.50
Zn & Cd	5.48	5.90	37.06
Cd & Pb--	n.d.	6.60	n.d.
Pb & Zn--			

- n.d. = not determined
-- Experiments ended as a result of lack of production of cocoons even in the control at t=21 days. This was attributed to high pH among other factors

3.2. EFFECT OF THE SINGLE METALS TO EISENIA ANDREI

First, data from the single toxicity test experiments are introduced (section 4.2.), because these data are necessary to interpret the combination toxicity studies, these are treated in section (4.3.).

3.2.1. **Growth and Mortality**

There was no pronounced dose effect relationship recorded for growth at different concentrations of the metals tested in all the single toxicity tests (figure 3 and appendix 1-5). Except for the fact that there was mortality of 2 worms, 6 worms and 5 worms at the highest concentration of Cd, Zn and Pb respectively. This implies that growth is not a sensitive parameter to estimate effects of these metals on Eisenia Andrei.

3.2.2. **Cocoon Production**

Figure 4, 5 and 6 summarises model fits of the dose effect relationships for cocoon production of Eisenia Andrei. The model parameters, and other information on model fits to these data, are summarized in Table 2.

The observed EC50 on cocoon production in the experiment with Cadmium was 103.9 mg/kg, at 102.4 – 105.5 95% confidence interval for this estimate. The percentage variance accounted for by the model was 84.4, which indicates an accurate fit of the model to the data.

In the experiment with Zn, when the data on cocoon production were fitted with the non-hormetic model, the EC50 observed was 279.21 mg/kg, and the percentage variance accounted for was 55.9. when fitted with hormetic model, the EC50 was 418.2 mg/kg, while the percentage variance accounted for was 85.2. moreover, hormesis was found to be significant ($P<0.001$). The presence of hormesis at low dose is indicative of the ability of the worms to be stimulated at the low doses of Zn to produce more cocoons than in the control.

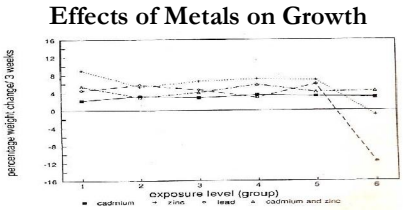

Figure 3. Dose effect relationship of Cadmium, Zinc, Lead and a mixture of Cadmium and Zinc on body growth of Eisenia andrei

With Pb, the EC50 could not be estimated by the model at the tested highest concentration of 1000 mg/kg dry soil in the first experiment. Hence, the experiment was repeated with higher concentration ranges and the EC50 was then estimated to be 1570mg/kg by the non-hormetic model. The percentage variance accounted for by this model was 68.5. This showed that the model gives an accurate description of the observations.

The number of cocoons per worm per week varied from 1.67 to 0.34 in the Cd experiment, 1.12 to 0.00 with Zn 0.80 to 0.00 with Pb (Appendix 1-4). For all metals, there was a clear effect of the dose of metals on the numbers of cocoons produced, since performance reduced by at least 50 percent in the exposed groups (Table 3); only with Zn and Pb there was no cocoon produced at the highest applied concentration. This means that the EC50 could be calculated by interpolation for all metals, which implies accurate estimation. Generally, it appeared that the accuracy of fitting of the model was higher for Cd, followed by Pb and then Zn.

The number of cocoons produced in the different experiments appeared to vary among experiments. This was demonstrated by the controls of each experiment. An average of 34 cocoons were produced in the control experiment with Cd, 33 in the control of Zn and 24 in the control of Pb (Appendix 1-4). Apparently, there is a factor which influences basic cocoon production, which might be either the physiological condition of the worms or the nitrate present. With additional information from the other experiments it is shown later (figure 12), that the number of cocoons decreased from the nitrate present in the control of experiment with Cd (upper left), via Pb (first experiment), Zn and Cd & Zn, towards Pb

(second experiment, lower right). The order of decrement shows that the number of cocoons produced decreased with increasing nitrate concentration. Since the model is normally used to fit the data of a single experiment, it would be improper to fit the model to the data of five separate experiments, as indicated in the figure.

Effect of Cadmium on Cocoon production

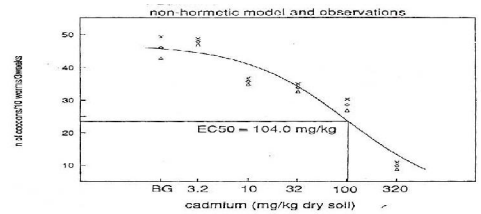

Figure 4. Dose-effect relationship of Cadmium exposure for cocoon in Eisenia andrei

Effect of Zinc on Cocoon Production

Hormetic model and observations

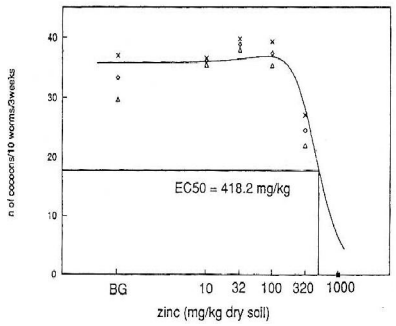

Figure 5. Dose-effect relationship of Zinc for cocoon production of Eisenia andrei

Effect of Lead on Cocoon Production

Non-Hormetic model and observations

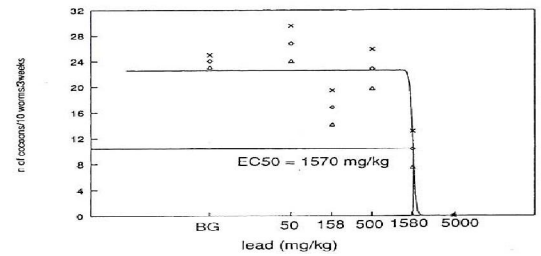

Figure 6. Dose-effect relationship for lead exposure for cocoon production in Eisenia andrei

3.2.3. Hatchability of Cocoons

The percentage for hatchability of cocoons were high in all tested concentrations of the metals, except for the highest concentration range of Zn and Pb, where no cocoons were produced and, therefore, no juveniles were found (Appendices 1-4). On the average, calculated over all concentrations within an experiment, the observed percentages hatchability were 92%, 95% and 93% for Cd, Zn and Pb, respectively. This indicates that, on the overall, the hatchability of the produced cocoons was not really affected by the metals.

3.2.4. Effects on Juveniles Production

There is slight difference between the number of juveniles produced per fertile cocoon in the control and the different tested concentrations of each metal in the experiments: the juvenile number per fertile cocoon decreased from 3.07 to 2.00 for Cd, 2.93 to 2.24 for Zn, 2.67 to 0.79 for Pb (Appendices 1-4). This implies that the metals do not interfere with the number of juveniles per cocoon.

Tables 2 and 3 summarize the model fits to data of juveniles production. The estimated EC50 of the effect of Cd on juveniles production was 28.51, at a 95% confidence interval of 28.51-29.82, by the non-hormetic model. The percentage variance accounted for was 89.4. apparently, juveniles numbers are more sensitive than cocoon numbers for Cd exposure (compare Fig. 4 and 7).

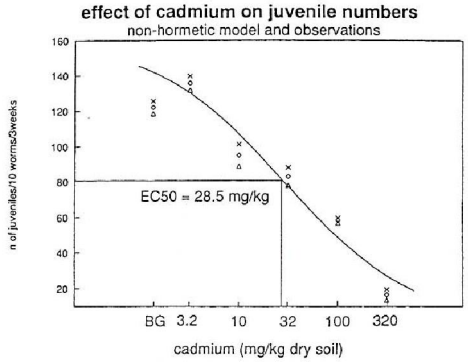

Figure 7. Cd exposure

For Zn, the estimated EC50 of its effect on juvenile production was 194.58mg/kg and the percentage variance accounted for was 59.5 with the non-hormetic model (Fig.8). With the hormetic model, the EC50 tended to infinity while the confidence interval could not be estimated; no variance could be accounted for by the model, and thus indicates bad fit of data. Within the same organism, it appears to be possible to observe hormesis in one reproduction characteristic (cocoon production), while it is lacking in the other (juvenile production).

In the experiment with Pb, the EC50 was 1034.4mg/kg with the non-hormetic model and the percentage variance accounted for by the model was 71.2 (Fig.9, Tables 2 and 3). Again, similar to Cd and Zn, juvenile production was more sensitive to metal exposure than cocoon production (compare Fig. 6 and 9).

Table 2. Summary of results of fitting models to toxicity data. Model: "N" indicates the non-hormetic model, "H" indicates the hormetic model, "%" indicates the percentage of variation accounted for by the model (fit), "B" indicates the slope parameter of both models, 'M' is the estimate of the ln(EC50), 'C' is the estimate of unstressed performance, 'F' indicates the hormetic parameter (if significant). The models were fitted both to the parameters C (Cocoon numbers) and J (juvenile numbers).

Substance and parameter	Model	%	B	M	C	F
Cd, C	N	86.6	-0.834	4.644	46.87	-
Cd, J	N	89.4	-0.659	3.350	160.90	-
Zn, C	N	55.9	-0.625	5.632	41.89	-
Zn, C	H1*	85.2	2.380	6.036	35.75	0.00064
Zn, C	H2*	18.1	1.091	13.82	1000.00	-
Zn, J	N	59.5	-0.669	5.270	114.40	-
Zn, J	H	-	-	-	-	-
Pb, C	N	68.5	-26.630	7.359	22.56	-
Pb, C	H	-	-	-	-	
Pb, J	N	71.2	-2.358	6.942	54.24	
Cd & Zn, C	N**	61.1	-11.832	0.946	27.13	
Cd & Zn, C	N***	76.7	-8.410	0.664	28.36	
Cd & Zn, J	N	65.9	-2.190	0.275	78.09	

- Hormetic model: the estimate of b in the non-hormetic model is equivalent to the estimate –b of the hormetic model, and indicates the slope at inflection (for further details: see text)

-- Model ran with defective cocoons inclusive

--- There are relatively high numbers of defective numbers only the highest conc. Since this affect in a major way the EC50 estimate, the programme was ran both with and without these cocoons.

Table 3. Summary of EC50 estimates (calculated from parameter M of the models) from fitting models to toxicity data (legends as in former table)

Substance	Parameter	Model	EC50(mg)	95% Confidence interval
Cd	Cocoon	N	103.96	102.42-105.53
	Juveniles	N	28.51	27.25-29.82
Zn	Cocoon	N	279.21	-
		H1*	418.2	-
		H2	(infinity)	(infinity)
	Juveniles	N	194.58	-
		H	-	-
Pb	Cocoon	N	1570.25	-
		H	-	-
	Juveniles	N	1034.40	-
Zn & Cd	Cocoon	N**	2.57(TU)	-
		N***	1.94(TU)	1.93-1.96
	Juveniles	N	1.32(TU)	1.30-1.33

- In this case, the hormetic model was run without (HI) and with (H2) defective cocoons. The model H2 did not show interpretable results.

-- Model run with defective cocoons observed particularly in the highest exposure concentration

--- Model run without the defective cocoons, which improved fit.

Based on the percentage variance accounted for by the models for different metals, the model fitted best data of the experiment with Cd, followed by that of Pb, and then Zn. Judged from the estimated EC50, Cd appeared to have a very strong effect on juvenile production, more than Zn and Pb, while Pb is least toxic to juvenile production.

Juvenile production appears to be a more sensitive parameter than cocoon production based on the estimated EC50 values of all the single toxicity experiments on both cocoon production and number of juveniles produced. The percentage variance accounted for by the model was always higher for effects on juvenile production than that of cocoon production except of Zn.

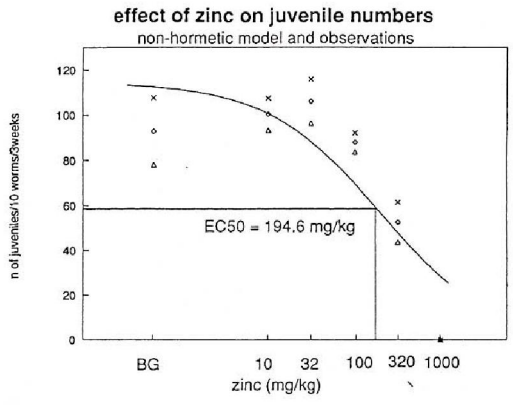

Figure 8. Dose-effect relationship of Zinc exposure for juvenile numbers in Eisenia andrei

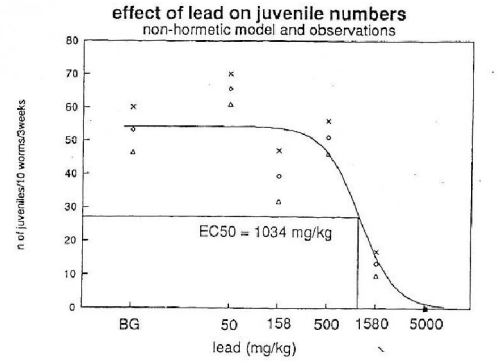

Figure 9. Dose-effect relationship of lead exposure for juvenile numbers in Eisenia andrei

3.3. COMBINATION TOXICITY EXPERIMENTS

3.3.1. Effect of a Cd & Zn Combination on Eisenia Andrei

Effects on Cocoon Production. Figure 10 summarises the dose-effect relationship of the effect of different concentrations of Zn & Cd combinations to cocoon production of E.andrei. at the highest two concentration ranges, an usually large numbers of defective cocoons was recorded. This may pose a problem to the interpretation of the combination toxicity test. To solve this, the model was run with one set of

data with defective cocoons included and the other with defectibe cocoons excluded.

The EC50 observed with data including the defective cocoons was 2.57 (TU) with the percentage variance accounted for being 61.1 (Tables 2 and 3). Without defective cocoons the EC50 was 1.94 (TU) with a confidence interval of 1.93-1.96 and a percentage variance accounted for 76.7 (Tables 2 and 3). From these TU values, it is clear that addition of the defective cocoons affects in a major way the TU obtained. The percentage variance accounted for by the data without the defective cocoons is higher that that of the one with defective cocoons. Based on the accuracy of fit, it is concluded that interpreting the data without adding the defective cocoons is better than with defective cocoons included.

Both TU values indicate that toxicity of the mixture of Cd and Zn is less than concentration additive on cocoon production of Eisenia Andrei, both for the good fitting model and the probably imperfect model fit. For further details, see Appendix 5 which summarized the original data for cocoon production of Eisenia Andrei exposed to an equitoxic combination of Zn and Cd.

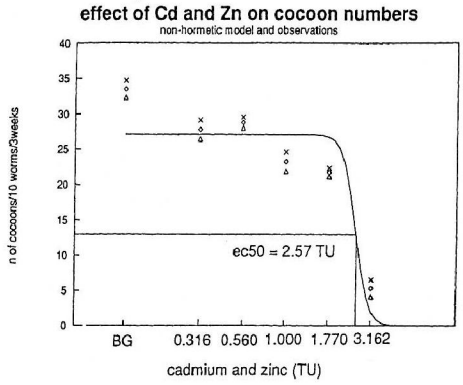

Figure 10. Dose-effect relationship of combined exposure to an equitoxic mixture of Cadmium and Zinc for Cocoon production in Eisenia Andrei

Effects on Hatchability of Cocoons. The percentage fertile cocoons for all the tested concentration ranges was on the average 94% (Appendix 5). This suggests that the hatchability of cocoons was not actually affected by the metal combinations, except for the recorded defective cocoons in concentration 6. the defective cocoons did not hatch, and were therefore, automatically, not influencing the model fits for juvenile numbers.

Effect on Juvenile Production. The mixture for combination toxicity test of Cd & Zn was prepared based on the EC50 of the single toxicity effect test of Cd and Zn on cocoon production of E.andrei, and not on their single toxicity tests results for juvenile production. This is because only one reproduction parameter can be studied in one combination toxicity test, and here major emphasis was put on the reproduction parameter cocoon production. Based on the EC50 values estimated for the single metals for juvenile production, TU were calculated as usual, but with the following principal difference for the interpretation. Since the equitoxicity was aimed to be valid for cocoon production alone, the principle of equitoxic concentrations was not applicable to the juvenile data. This means, that the mixture contained relatively more Zn than Cd. After calculation of the soil concentrations prepared, and estimating the TUs for juvenile numbers, it appeared that the ratio of concentrations of Cd and Zn in this mixture, expressed as toxic units, were 2.168, and not 1 as usual in an equitoxic mixture. The estimated EC50 from the non-hormetic model is 4.46 TU (Fig. 11, Table 2,3), this is equivalent to a mixture of Cd and Zn with concentrations of 87.0mg/kg of Cd and 274.0 mg/kg Zn. Apparently, much more Cd and Zn are needed than expected from the input data to obtain an EC50 effect. This indicates, again, an antagonistic effect between Cd and Zn.

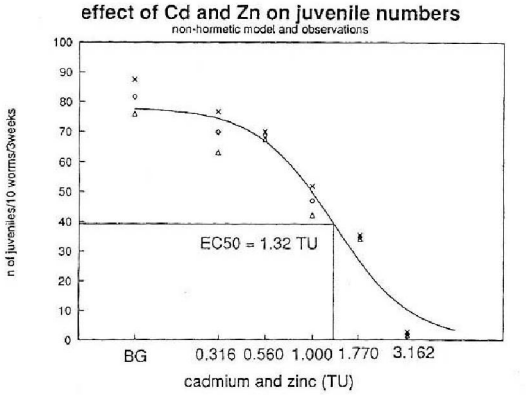

Figure 11. Dose-effect relationship of exposure to a mixture of Cadmium and Zinc for juvenile numbers in Eisenia Andrei. For further explanation: see text.

When judged by the EC50 of the single metals, the relative toxicities of Cd and Zn on juvenile production can be compared. Cd appeared to be 11 times more toxic than Zn for juvenile production.

3.3.2. Effects of Cd & Pb and Zn & Pb Combinations on Eisenia Andrei

With Pb & Zn and Pb & Cd, no scientifically reasonable data of the effect of the metal production to E. Andrei could be gathered. This is because at the end of 3-week exposure period of the worms to the metal combination of Pb & Cd, no cocoons were observed in the control of the experiment and only 1 cocoon was recorded for concentration number 3. hence, the result of the experiment was disregarded as far as this combination toxicity research is concerned. The test on Pb & Zn was also prematurely stopped after checking

each replicate of the 6 concentration at the second-week of the duration of the experiment. This was due to the alarm raised by the result of the former combination test. In both of the experiments, the number of cocoons produced in the acclimation phase were on the average 7 versus an average of 13 recorded in previous tests

4. DISCUSSION

4.1. SINGLE TOXICITY TEST

Cd had the most adverse effect on cocoon production, followed by Zn and Pb. Similar results have been recorded on the effects of Cd and Zn on this parameter. Both Van GEstel (1991) and Spurgeon et al. (1993) observed a similar order of toxicity of Cd, Zn and Pb on cocoon production of Eisenia Andrei (Savigny). So, the present results for single toxicants are in good agreements with previous work by other authors. Therefore, the above order of relative toxicity should be taken to be scientifically reliable.

The numbers of juveniles produced were generally more affected by the individual metals than the numbers of cocoons produced. This is judged from the EC50 of the effect of the metals on production. This experiment also supports the observation of Spurgeon et al. (1993) that juvenile number is a more important parameter for toxicity test of metals, than cocoon production.

Growth was not affected by the metals at all concentrations tested, except for the highest concentration of Pb. There was a slight increase in weight of the worms except for the highest concentration of Zn, and in the experiment with Pb Growth appeared to be an insensitive parameter for toxicity tests Cadmium, Zinc and Lead in earthworms. Therefore, growth may not be an important parameter for calculation of data for combination toxicity tests of Cd, Zn and Pb in earthworms tests.

The largest numbers of cocoons were recorded for the control of Cd, then Zn, then Pb. This could potentially be attributed to the biological differences of batches of worms, and to the effects of nitrate. Since figure 12 shows that there is dose effect relationship of nitrate concentration to cocoon production, I conclude that nitrate influenced the number of cocoons produced. In future, equal concentrations of nitrate should be maintained in all the tested metals as a whole, and not just within the different concentrations of each metal. This is already applied by Weltje (RIVM, 1993) in further experiments with earthworms exposed to heavy metals (pers, comm.., Lenmnart Weltje).

4.2. COMBINATION TOXICITY TESTS

The present study forms a landmark in the combination toxicity studies of heavy metals using earthworms. However, information is generally lacking on the combination toxicity effects of heavy metals to terrestrial organisms. It is only in aquatic literature that extensive studies have been recorded on this subject. First, therefore, some comments will be made on the results from aquatic literature (4.2.1.), followed by an evaluation of the present results (4.3.2.)

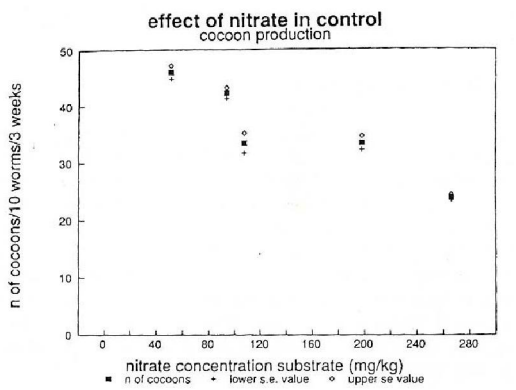

Figure 12. Relationship between amounts of nitrate in controls of (left to right) exp. With Cd, Pb(1), Zn, Cd & Zn, Pb(2) and cocoon production in Eisenia Andrei

4.2.1. Information From Aquatic Literature

The EIFAC (1987) presented a literature review on combined effects on freshwater fish and other aquatic life forms of mixtures of toxicants in water. Based on the report, the action of Cu and Zn appeared to be several-fold more than additive on the percentage survival of mummichog in synthetic sea water (20% salinity). The presentation reported conflicting result of combination of Cu and Cd to mummichog, baed on different parameters. Whereas Cu and Cd appeared to be less than concentration additive on their effect on the lateral lines of mummichog, the two metals have also been reported to be more than additive on both a response and concentration basis to survival of mummichog in synthetic sea water. Lethal effects of Cu and Cd in mixtures were found to be almost 2-fold more than additive to zebra fish in 96-hour acute tests.

Kraak (1992) evaluated the effects of equitoxic mixtures of Cu, Zn and Cd on the filtration rate of the freshwater mussel (Dreissena polymorpha) using the toxic unit concept, and he found that the effect of a mixture could not be predicted from the effects of the single metals. Cu and Cd contributed to the toxicity of a mixture of Cu, Zd and Cu at or below the NOEC for these metals, determined in single metal toxicity test, indicating concentration addition and potentiation, respectively.

Kraak (1992) also studied the chronic ecotoxicity of mixtures of cu, Zn amd Cd to the zebra mussel Dreissena polymorpha. In short-term experiments, Cu + Cd were strongly more than additive, indicating a loss of potential for additivity during prolonged exposure. It was concluded that the chronic effects of mixtures could neither be predicted from their short-term effects, nor from the chronic effects of the metals tested individual.

Enserink et al. (1991) did an ecological evaluation of reports on combined effects of metals. In their work, As, Cd, Cu, Hg, Pb, Ni and Zn were tested singly and in equitoxic mixtures based on the LC50 (individual Daphnia magna) or EC50 (population) of single metals. The result of their work showed nearly concentration addition of the metal mixture to individual survival as well as population growth of Daphnia magna in most cases.

Spehar and Fiandt (1986) worked on acute and chronic effects of water quality criteria-based metal mixtures on three aquatic species. In their work, As, Cd, Cr, Cu, Hg and Pb combined at criterium concentrations caused nearly 100% mortality in rainbow trout and daphnids (Ceriodaphnia dubia) during acute exposure. Fat-head minnows were not adversely affected at this or at two times this concentration, although a mixture of four to eight times the maximum values caused 15 to 60% mortality. This shows that effects of different metal combination differ from one organism to the other. Metals combined at the criterion average concentrations significantly reduced production of daphnid young and growth of fathead minnows after 7 and 32 days, respectively. In their further work, acute tests with metals at multiples of LC50 concentrations indicated that the joint action of the metals was more than additive for daphnids, based on toxic units calculated from the individual components of the mixture. Chronic tests showed that the joint action was less than additive for fathead minnows but nearly strictly additive for dapnids, indicating that long-term metal interationc may be different in fish compared to lower invertebrates. This result clearly pointed out that single chemical derived water quality criteria may not sufficiently protect some species when other toxicants are also present.

To summarise, experiment results available on combination toxicity in aquatic ecotoxicology show some contradicting results at first sight. Toxicity effects resulting from different combinations of metals differ with different parameters and different experimental conditions and duration of experiment. Despite this snag, the fact that combination toxicity experiments reflects the actual pollution of aquatic ecosystems in a more realistic way than experiments in which toxicants are tested individually, has been emphasized by many authors.

4.2.2. **Information From Experiments With Terrestrial Organisms**

Focusing on metal combination in soil, Bewley and Stotky (1983) investigated the effects of combinations of Cd and Zn on microbial activity in soil and the influence of clay minerals when metals were added simultaneously. They found that the effect on the lag period for population growth of the two metals together was concentration additive.

The EC50 for cocoon production in earthworms exposed to an equitoxic mixture of Cd and Zn was estimated to be 1.94 TU. Based on the concept of the Toxic Unit model, this result can be interpreted to mean that the metals are less than additive (antagonistic) (Sprague, 1970). Since the results of the combination toxicity experiment of Zn & Pb and Pb & Cd could not be obtained in this research, the effect of different mixture on cocoon production and juvenile production can not be compared favourably with that of their single toxicity.

The lack of observations for cocoon production was attributed to some possible factors:

1. The high pH value, as the pH read at the end of the experiment were 6.6 for Pb & Cd and 6.5 for Pb & Zn, versus the highest value of 5.0 recorded in previous experiments.
2. The weight of the works were on the average of 0.295 and 0.283 as against 0.35 and 0.37 recorded on average in previous tests.
3. May be that there was an unidentified adverse environmental factor that interfered with the experiment.

No previous or further information is available in terrestrial literature on effects of metal combinations to earthworms. If it should be taken that the two metals are antagonistic, it can be explained by the fact that the ions may complete for target molecules. In the presence of a high amount of Zn (a high number of molecules), Cd may not easily reach the target, so that toxicity decreases, in that case, Cd, which has been proved to be more toxic than Zn in single toxicity test, is rendered less toxic when acting in equitoxic concentration with Zn. It should be reminded that Zn is an essential element (micronutrient), whereas Cd is completely non-essential to any form of life.

4.3. **ECOLOGICAL TRANSLATION TO NATURAL CONDITIONS**

In the aquatic environment, discrepancies found between additive action and more than additive action of metals in organisms may be due to water quality characteristic, such as water hardness, that may alter metallic forms (EIFAC, 1997). In natural soils, similarly, different soil characteristics may also influence the toxicity of mixtures of chemicals to soil organisms. There is, however, no information available in literature to support this point. Mixtures of two substances have been found to be less than concentration additive in most cases, whereas mixtures of up to six substances are usually concentration additive (Spehar et al. 1970). In polluted sites, there would exist the probability of a Cd & Zn mixture being concentration additive in the presence of other metals.

It was observed that the metals did not interfere with the hatchability of the cocoons or with the number of juveniles per fertile cocoon. It could be possible that such an adverse effects of metals on juveniles could be

observed in natural conditions, where the juveniles are exposed from hatching, in contrast to the laboratory situation. There may exist a difference in the survival and fitness of the juvenile if tested in a complete life cycle test.

Since E.andrei still acts like their aquatic ancestors (Edward & Lofty, 1977) it is recommended to carry out toxicity tests for the soil compartment with other important and completely terrestrial soil organisms in addition, like springtails from the upper litter layer of the soil, isopods, centipedes or millipedes, in order to obtain more reliable information on combination toxicity effects to terrestrial organisms in the soil compartment.

More experiments is need in this area for a favourable comparison of available information in aquatic literature on combination toxicity with results of similar studies with terrestrial organism.

4.4. CONCLUSION AND RECOMMENDATIONS

The model of combination toxicity of mixtures has been shown by this research to be applicable to E.andrei exposed to equitoxic mixtures of Zn & Cd. The toxicity of Cd to the reproduction of E. Andrei appeared to have been reduced by the presence of equitoxic concentration of Zn, or the other way round.

The result of the present study suggests that the toxicity of combination of metals might not be predicted from the EC50 of the single metals to the same parameter. Hence, single toxicity tests alone may not determine the fate of earthworms in sites polluted with combinations of chemicals.

This study demonstrates that the effect of Cd, Zn and Pb to cocoon production in the single toxicity test are in agreement with that of other authors like Van Gestel (1991) and Spurgeon et al. (1993).

For all the single toxicity test experiments, cocoon production and number of juveniles emerging from the produced cocoons appeared to be important basic data for calculation of mixtures for combination toxicity test.

The use of EC50 of 'number of juveniles' produced is recommended as basic data for calculation of mixture concentrations for combination toxicity tests when effect of metals on the life cycle of E.andrei are to be carried out. This will give a very good indication of the effect of the metals to the survival and fitness of the resultant juveniles. This will in turn give a better indication of long term effect of toxicants like metals to earthworms.

More experiments are needed in the area of terrestrial ecotoxicology on the mixture effects of metals, to enable a favourable comparison of the obtained results with available information in aquatic literature.

In future, abiotic factor, such as pH and humidity of OECD medium should be checked before starting of toxicity experiments. This is contrasting to the presently applied approach of measuring these abiotic factors after exposure of worms. Similar batches of different components of artificial soil substrates should be used throughout a research. These additional precautions will help to exclude possibilities of variation of environmental conditions in a particular research phase.

Equal amounts of nitrate should always be maintained in all the concentrations of metals tested, instead of within each series of concentrations for each tested metal separately. This is meant to adjust for the influence of nitrate on dose-effect relationships of metal salts to cocoon production.

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Bacillus Calmette-Guérin versus Gemcitabine for Intravesical Therapy in Intermediate and High-Risk Superficial Bladder Cancer

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Abstract: Bacillus Calmette Guerin (BCG) has been the mainstay of intravesical treatment, however, its clinical effectiveness is accompanied by a wide range of adverse events. Gemcitabine has a good safety profile with promising features for the use against intermediate risk non-muscle invasive bladder cancer (NMIBC). It can be a potential chemotherapeutic drug for high- risk patients. The aim of this study was to evaluate the safety and efficacy of adjuvant intravesical gemcitabine versus BCG in the treatment of intermediate and high -risk NMIBC. Patients and methods: Between May 2006 and April 2008, a total of 57 patients were randomized into 2 groups; group I: 28 patients, were treated with six weekly intravesical instillation of BCG and group II: 29 patients, received six weekly intravesical grmcitabine. Patients were evaluated for response, at 8 weeks, then every 3 months. Outcome measures were response rate, overall recurrence rate, progression rate, median recurrence free period, median progression free period and 1-year recurrence free survival. Treatment related complications were also evaluated. Results: For intermediate risk patients, there was no significant difference between the two groups in the complete response (CR) rate (93.3% vs. 87.5%), the overall recurrence rate (33.3%vs.25%), the progression rate (6.7% vs. 6.2%), and the median progression free period (13 vs. 16 months). However, the median recurrence free period was longer for group I compared to group II (18.5 vs. 15 months) and the difference was statistically significant. Kaplan-Meier curve showed that there was no significant difference between the two groups in the 1-year recurrence free survival (95.3% vs. 98.7%) and the median recurrence free survival (22 vs.18 months). For high risk patients there was no significant difference between the 2groups in CR rate (61.5% vs. 76.9%), the progression rate (15.4% for both groups) the median recurrence free period (15 vs. 14 months) and the median progression free period (17 vs. 15 months). However, the overall recurrence rate of group I was lower than that of group II (7.7% vs. 30.8%) and the difference was statistically significant. Kaplan-Meier curve showed that there was no significant difference between the two groups in the 1-year recurrence free survival (76.9% vs. 69.2%) and the median recurrence free survival (18 vs.15 months). The adverse events of group I were more marked than that of group II. Conclusion: Gemcitabine is active and well tolerated for intravesical instillation.It is considered to be an efficient treatment for intermediate risk NMIBC. However, for high- risk group, it is inferior to BCG, but owing to its favorable toxicity profile, it may be useful for patients intolerant to BCG.

[Ahmed M. Abd-Alrahim and Hoda H. Essa, Bacillus Calmette-Guérin versus Gemcitabine for Intravesical Therapy in Intermediate and High-Risk Superficial Bladder Cancer]. Journal of American Science 2011;7(8):416-426]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Bacillus Calmette-Guérin; Gemcitabine; Intravesical Therapy; Bladder Cancer

1. Introduction

Transitional cell carcinoma (TCC) is the second most common urologic malignancy, and 70% of patients present with superficial, or non-muscle invasive disease (NMIBC)[1]. The probability of recurrence is about 60% within one year and 80% within five years. Risk of tumour progression to muscle invasion is about 17% to 45% in one to five years respectively [2]. ../Administrator/My Documents/Downloads/paper0/article.asp.htm -ref1Chemotherapy and immunotherapy with bacillus Calmette-Gue´rin (BCG); are the main forms of intravesical instillation therapy following transurethral resection (TURBT)[3]. The goals of intravesical therapy are to avoid post-TURB implantation of tumour cells, eradicate residual disease, prevent tumour recurrence, and delay or reduce tumour progression[4]. Chemotherapy reduces recurrence frequency and, therefore, further resection requirements, and BCG treatment is reported to delay the progression of high-risk tumours[5].

The intermediate-risk disease can be treated with either immunotherapy with BCG or chemotherapy, and BCG is now the treatment of choice for high-risk tumours[6]. Unfortunately, approximately 20% of patients discontinue BCG due to local and systemic toxicity and more than 30% show evidence of recurrence; this has led to increased interest in alternate chemotherapeutic agents. Induction intravesical chemotherapy has shown comparable efficacy to BCG in select patients and the immediate perioperative instillation of chemotherapeutic agents has become standard of

care[1].

Gemcitabine is a novel deoxycytidine analogue with a broad spectrum of anti-tumour activity. Its pharmacokinetic properties also make gemcitabine an ideal candidate for regional therapy. Gemcitabine seems to have fulfilled the requirements to be a promising new candidate for standard intravesical therapy in NMIBC[7].

2. PATIENTS AND METHODS

Inclusion and exclusion criteria:

Patients with NMIBC scheduled to undergo curative resection by TURBT at the department of Urology, South Egypt Cancer Institute and oncology department, Assiut University hospital were enrolled into this prospective study. Between May 2006 to April 2008 a total of 69 patients were involved in this study.The cutoff date of the analysis of the outcome was April 2008 ,corresponding to 1 year of follow up for the last patient enrolled in the study.Patients were randomized into 2 groups;35 patients in group I and 34 patients in group II. However, 7 patients in group I discontued treatment primarily because of marked treatment toxicity and 5 patients in group II discontued treatment;1 patient due to severe haematurea and the other 4 patients were missed due to unknown causes. Eligible patients were those with intermediate and high-risk NMIBC, based on the European Organization for Research and Treatment of Cancer scoring system(EORTC) [2]. Intermediate risk patients included: multiple grade 1 stage T1 tumours, grade 2 stage Ta tumours and a single grade 2 stage T1 tumours. High risk patients included: multiple grade 2 stage T1 tumours, grade 3 stages Ta and T1 tumours, and carcinoma in situ. Informed consent, a WHO performance status of 0–2, WBC > 3000, PLT > 100.000, Hb_> 10 g/dl, renal and hepatic function values not exceeding 2 times the upper normal value were also required for study entry. Patients were excluded from the study if they were considered to have low-risk NMIBC i.e. single grade 1,2 stage Ta tumour , or had any other severe illness. Concomitant or recurrent urinary tract infections and the presence of significant urological disease interfering with intravesical therapy constituted exclusion criteria. Patients were enrolled consecutively to the study and randomized to either group I or group II. The pre-treatment assessments included a full medical history and examination, plain chest X ray, urine culture, full blood count, and liver and kidney function.

Treatment schedule and toxicity monitoring:

Group I patients received six weekly intravesical instillations of BCG (Pasteur strain) 150 mg in 50 ml saline. Group II received intravesical instillations of 2000 mg of gemcitabine in 50 ml of normal saline (0.9%) with a final concentration of 40 mg/ ml. The pH of the reconstituted solution varied between 2 and 3 and no buffering was adopted. The patients were asked to avoid urinating for 1 hour after the instillation. Protocol therapy consisted of 6 weekly instillations to be started within 15 days of the TUR. Urine cytology, urine culture, full blood count, and liver and renal function were assessed.

Toxicity was assessed with the use of the Common Toxicity Criteria version 3.0 Table (4-a) [8]. Grade 3 side-effects resulted in patients' exclusion from the study. In case of grade 2 toxicity, the treatment was delayed for 1 wk and repeated. If toxicity relapsed at grade 2, the treatment was stopped. Side-effects were checked after each instillation and recorded in the database.

Evaluation:

Outcome measures were response status, recurrence status and overall recurrence rate, progression status and overall progression rate, median recurrence free period and progression free period. 1-year recurrence free survival,median recurrence free survival and treatment related complications were also evaluated. Patients' response status was defined at cystoscopy as complete responders (CRs; i.e., absence of any macroscopic residual lesion, confirmed by negative histology and cytology) and non responders (NRs; i.e., presence of any residual lesion). Recurrence was determined by lesions that were detected at cystoscopy and pathologically confirmed after TUR, and Progression was defined as an increase in tumour stage and grade. The recurrence and the progression rate was defined as the percentage of recurring or progressing patients at 1-year follow-up. Recurrence free period was defined as the time from TUR to the date of the first recurrence and the progression free period was defined as the time between TUR and first progression. 1-year recurrence free survival was defined as the time from the date of TUR to the date of recurrence or last follow-up among patients who achieved a CR at 1-year follow-up.

Statistical analysis:

Data were recorded on specialized forms and all statistical tests were performed using SPSS version 16 for windows (SPSS Inc, Chicago, IL, USA) and Microsoft Excell (Realmond, W.A, USA) software. Descriptive analysis (e.g., mean, median, standard deviation, frequencies, percentage) were calculated and analysis was performed using the student's t-test and Fisher ExactT- Test, P value <0.05 was considered significant. The survival curves were made using the Kaplan-Meier method

and comparison was with the log rank test.

3. Results

Patients characteristics: table (1)

The median age of patients of group I was 60 years range (45-82 years) while it was 57 years (range 30 – 78 years) for group II. There were 21 men (75%) and 7 women (25%) in group I with male: female ratio of 3:1, while there were 24 men (82.8%) and 5 women (17.2%) in group II with male: Female ratio of 4.8:1 for group II. On stratification of the patients according to risk, 15 patients (53.6%) in group I were of intermediate risk and 13 (46.4%) were of high-risk, while 16 patients (55.2%) of group II were of intermediate risk and 13 patients (44.8%) were of high –risk as shown in table(1).

Outcome:

The minimum period for follow up was 12 months for both groups,while the maximum period was 30 and 29 months and the median period of follow up was 17.5 and 16 months for group I and II respectively. Twenty two patients (78.6%) of group I achieved CR, while 24 patients (82.8%) of group II had CR, with no significant statistical difference (p=0.47). The overall recurrence rate of group I was 21.4% (6 patients), while it was 27.6% (8 patients) for group II, with no significant statistical difference (p=0.7). The progression rate was 10.7% and 10.3% for group I and II respectively (3 patients for both groups) with no significant statistical difference (p=0.5).The minimum period of time to recurrence was 10 and 3 months and the maximum period was 30 and 29 months for group I and II respectively. The median recurrence free period was 14 and 15 months for group I and II respectively with no significant statistical difference (p=0.2).The minimum period of time to progression was 3 months for both groups while the maximum period was 30 and 29 months for group I and II respectively. The median progression free period was 18 and 16 months for group I and II respectively with no significant statistical difference (p= 0.9) as shown in table (2).

Kaplan-Meier curves showed that the l-year recurrence free survival rate was 88.1% and 96.6% and the median recurrence free survival was 19 and 16 months for group I and II respectively, with no statistical significance (p= 0.7) (Fig. 1).

When patients were stratified according to risk, It was found that for intermediate risk patients, the CR rate was 93.3% and 87.5% (14 patients for both groups) for group I and II respectively with no statistical significance (p= 0.5). The overall recurrence rate was slightly higher in group I (33.3%; 5 patients) compared with group II (25%; 4 patients) but this difference was not statistically significant (p=0.3). The minimum period of time to recurrence was 12 and 3months and the maximum period was 25 and 18 months for group I and II respectively.The median recurrence free period was longer for intermediate risk patients of group I (18.5 months) compared with those of group II (15 months), and this difference was statistically significant (p=0.005). The progression rate was nearly similar (6.7% and 6.2%) for group I and II respectively (1 patient for both groups) with no significant statistical difference (p= 0.6). The minimum period of time to progression was 10 and 6 months while the maximum period was 25 and 26 months for group I and II respectively.The median progression free period was 13 and 16 months for group I and II respectively with no significant statistical difference (p= 0.8) as shown in table (3).

Kaplan-Meier curves showed that the 1-year recurrence free survival rate was nearly similar for the intermediate risk patients of both groups (95.3% and 98.7%) and the median recurrence free survival was 22 and 18 months for group I and II respectively with no significant statistical difference (p=0.6) (Fig. 2).

For high risk patients, the CR rate was 61.5% (8 patients) for group I and 76.9% (10 patients) for group II with no significant statistical difference (p= 0.3). The overall recurrence rate was 7.7% (1 patient) in group I, while it was 30.8% (4 patients) in group II and this difference was statistically significant (p=0.04). The minimum period of time to recurrence was 12 and 3months and the maximum period was 30 and 28 months for group I and II respectively. The median recurrence free period was 15 and 14 months for group I and II respectively with no significant statistical difference (p=0.1). The progression rate was 15.4% (2 patients) for both groups with no significant statistical difference (p= 0.5). The minimum period of time to progression was 3 months for both groups while the maximum period of time to progression was 30 and 28 months for group I and II respectively. The median progression free period was longer for group I (17 months) compared with that of group II (15 months) but the difference was statistically not significant (p= 0.7) as shown in table (3).

Kaplan-Meier curves showed that the 1-year recurrence free survival rate was 76.9% for high risk patients of group I, while it was 69.2% for group II, and the median recurrence free survival was 18 and 15 months for both groups respectively and the difference was statistically not significant (p= 0.08) Fig. (3).

Comparison of the local side effects showed overall, few severe (grade 3) adverse events in the 2 treatment groups. Dysurea was the most frequent

local side effect in group I constituting 46.4% (13 patients) with grade 3 occurred in 3 patients, while it constituted 6.9% (2 patients) in group II with only 1 patient had grade 3 and the difference was statistically significant (p=0.001). Haematurea was the next most frequent side effect in group I constituting 32.1% (9 patients) with grade 3 occurred in 2 patients while it was 13.8% in group II (4 patients) and the difference was not statistically significant (p= 0.09). Urinary frequency was another local complaint, described by 7.1% (2 patients) in group I and 10.3% (3 patients) in group II and the difference was not statistically significant (p=0.5). As regards the systemic side effects, fever was the main side effect in group I constituting 17.9% (5 patients) with only 1 patient had grade 3, while there were no systemic side effects in group II. table (4-b), Fig. (4).

Table 1: Patient characteristics of the 57 patients:

		Group 1 Intravesical BCG (n=28)	Group 2 Intravesical gemcitabine (n = 29)	
Sex	Male	21(75.0%)	24(82.8%)	0.5*
	Female	7(25.0%)	5(17.2%)	
Age		60	57	0.02**
Multifocality		10(35.7%)	13(44.8%)	0.6*
Site	Ant.	1(3.6%)	2(6.9%)	N/A
	Post.	17(60.7%)	15 (51.7%)	
	Ant.& post.	2(7.1%)	7(24.1%)	
	Left	6(21.4%)	4(13.8%)	
	Right	1(3.6%)	0(0.0%)	
	Dome	1(3.6%)	0(0.0%)	
	Ant.& left	0(0.0%)	1(3.4%)	
Stage	Ta	9(32.1%)	7(24.1%)	0.4*
	T1	19(67.9%)	22(75.9%)	
Grade	G1	8(28.6%)	14(48.3%)	0.1*
	G2	7(25.0%)	2(6.9%)	
	G3	13(46.4%)	13(44.8%)	
Risk	Intermediate	15(53.6%)	16(55.2%)	0.6*
	High	13(46.4%)	13(44.8%)	

*Fisher Exact test & **Independent T-test

Table (2) : treatment outcome of the 2 groups:

	Group 1 Intravesical BCG (n=28)	Group 2 Intravesical gemcitabine (n=29)	p-value`
CR	22(78.6%)	24(82.8%)	0.5*
Recurrence	6(21.4%)	8(27.6%)	0.7*
Progression	3(10.7%)	3(10.3%)	0.5*
Median recurrence free period (months)	14	15	0.2**
Median progression free period(months)	18	16	0.9**

*Fisher Exact test & **Independent T-test

Table (3): treatment outcome of the 57 patients according to risk groups:

Variables		Group 1 Intravesical BCG (n=15)	Group 2 intravesical gemcitabine (n = 16)	p-value
Intermediate Risk	CR	14(93.3%)	14(87.5%)	0.5*
	Recurrence	5(33.3%)	4(25.0%)	0.3*
	Progression`	1(6.7%)	1(6.2%)	0.6*
	Median recurrence free period (months)	18.5	15	0.005**
	Median progression free period (months)	13	16	0.8**
High Risk (n=13)	CR	8(61.5%)	10(76.9%)	0.3*
	Recurrence	1(7.7%)	4(30.8%)	0.04*
	Progression`	2(15.4%)	2(15.4%)	0.5*
	Median recurrence free period (months)	15	14	0.1**
	Median progression free period(months)	17	15	0.7**

*Fisher Exact Test & ** Independent T-test

Table (4-a): Toxicity grades according to Common Toxicity Criteria version 3.0:

	Grade 1	Grade 2	Grade 3	Grade 4
Dysurea	Asymptomatic	Frequency with dysuria; macroscopic hematuria	Transfusion; IV pain medications; bladder irrigation indicated	Catastrophic bleeding; major non-elective intervention indicated
Heamaturea	Minimal or microscopic bleeding; intervention not indicated	Gross bleeding, medical intervention, or urinary tract irrigation indicated	Transfusion, interventional radiology, endoscopic, or operative intervention indicated; radiation therapy (i.e., hemostasis of bleeding site)	Life-threatening consequences; major urgent intervention indicated
Frequency	Increase in frequency or nocturia up to 2 x normal; enuresis	Increase>2 x normal but<hourly	≥1 x/hr; urgency; catheter indicated	—
Fever	38.0 – 39.0°C	>39.0 – 40.0°C	>40.0°C for ≤24 hrs	>40.0°C for >24 hrs

Table (4 -b): treatment toxicity of the 2 groups:

	Group 1 Intravesical BCG (n=28)	Group 2 Intravesical gemcitabine (n=29)	P-value*
Dysurea	13(46.4%)	2(6.9%)	0.001
Grade 1	-	-	
Grade 2	10	1	
Grade 3	3	1	
Grade 4	-	-	
Heamaturea	9(32.1%)	4(13.8%)	0.09
Grade 1	-	1	
Grade 2	7	3	
Grade 3	2	-	
Grade 4	-	-	
Frequency	2(7.1%)	3(10.3%)	0.5
Grade 1	2	1	
Grade 2	-	2	
Grade 3	-	-	
Grade 4	-	-	
Fever	5(17.9%)	0(0%)	0.001
Grade 1	1	-	
Grade 2	3	-	
Grade 3	1	-	
Grade 4	-	-	

*Fisher Exact test

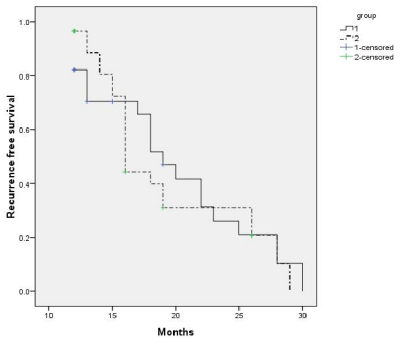

Figure (1): The recurrence free survival of the 2 groups of patients: Kaplan–Meier curves showing a non-significant difference in recurrence free survival between patients with NMIBC treated with intravesical BCG (group I) and patients who received gemcitabine (group II).

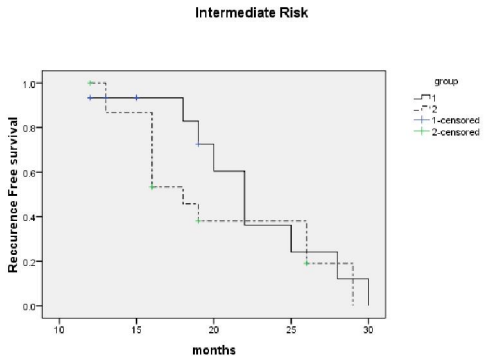

Figure (3): The recurrence free survival of the high risk group: Kaplan–Meier curves showing a non significant difference in recurrence free survival between patients with intermediate risk NMIBC treated with intravesical BCG (group I) and patients who received gemcitabine (group II)

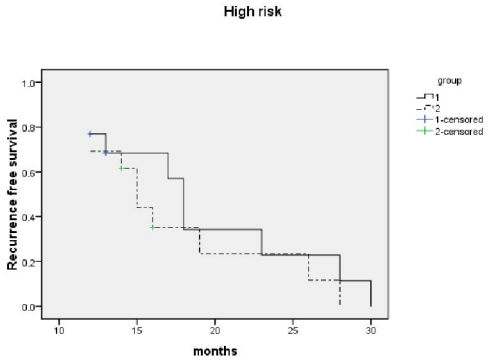

Figure (3): The recurrence free survival of the high risk group: Kaplan–Meier curves showing a non significant difference in recurrence free survival between patients with intermediate risk NMIBC treated with intravesical BCG (group I) and patients who received gemcitabine (group II)

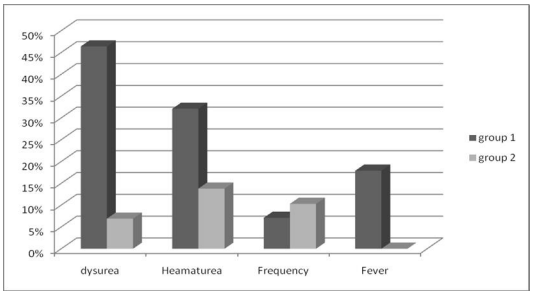

Figure (4): treatment toxicity of the 2 groups

4. Discussion

The advent of effective and safe intravesical therapies has improved the management of NMIBC, however, there is still clear need for novel or improved adjuvant treatment modalities [9].

There are limitations in the efficacy of intravesical treatment for intermediate and high-risk NMIBC. Though intravesical adjuvant therapy with BCG is superior to any other immunotherapeutic/chemotherapeutic agent in reducing tumour recurrence and disease progression, its real efficacy remains controversial as one-third of the patients will be non-responders. Also, following conventional intravesical chemotherapy, the short term recurrence rate of intermediate risk NMIBC cannot be reduced by more than 15-20% and long term risk of recurrence by 6% [10]. Hence, there is increasing interest in alternative first-line drugs for the treatment of the intermediate and high-risk NMIBC[11,4].The aim of this study was to evaluate the safety and efficacy of adjuvant intravesical gemcitabine versus BCG in the treatment of intermediate and high-risk NMIBC.

Intravesical gemcitabine has been tested in several phase II studies that explored its clinical utility for intermediate and high-risk NMIBC. Activity against marker lesions and primary and previous refractory tumours has been demonstrated in multiple trials.

In the present study, the CR rate of gemcitabine arm is comparable to that of the BCG arm but higher than that reported by others. Phase II studies have showed CR in up to 60% of cases of marker lesions in intermediate risk and few attempts have been made to test the activity of intravesical gemcitabine in high risk group [12, 13, 11, 14]. Dalbagni et al., tested the efficacy of intravesical gemcitabine in patients with BCG refractory, high risk NMIBC in a phase II prospective trial. Results showed that 50% of patients had a CR and 21% were free of disease at one year [15].

The CR rate of BCG arm is comparable if not better than that reported in previous similar studies where it ranged from 35% to 84% [16,17,18,19]and for high risk group the CR ranged from 64% to 84% [20,21,22,23]. The variation between this study and the other series can be explained by the presence of large numbers of patients with lower risk tumours and by variation of regimens and dosages used where most of those regimens include maintenance.

In the present study,The recurrence rate of the gemcitabine arm is comparable to that of BCG arm, however, there is a significant reduction in recurrence in favor of BCG in the subgroup analysis involving patients with high risk group. The recurrence rate of gemcitabine arm is comparable to that of Bartoletti et al., where they reported a recurrence rate of 25.4% while the 1- year recurrence free survival was 74.6% [24]. In the study of El-Koushy, intravesical gemcitabine was tested in patients refractory to BCG, recurrence rate was 28.6% [25].

The recurrence rate of BCG arm is lower than that of Elmallah, (29.6%) Lamm, (31%), Lundholm (51%), Bohel et al., (40%), Kim et al., (26.7%) and Cho et al., (33%) [26, 27, 28, 29, 3,5].conversely, it is higher than that of librenjack et al., (12%).[30].

With regard to disease progression, the results of BCG versus chemotherapy are less clear [31]. It has been proved that BCG decreases the progression of superficial bladder cancer, however a meta-analysis of EORTC and medical research council data demonstrated that chemotherapy prevents recurrence but not progression [32,33]. Another meta-analyses showed that chemotherapy delays the time to first recurrence, however it has not been shown to influence either the time to progression to muscle invasive disease, duration of survival or progression free survival [31]. In our study, the progression rate of the gemcitabine arm is

comparable to that of BCG arm. This result is parallel to that of Lundholm et al., and Krege et al., on their comparative study between BCG and MMC, where tumour progression was similar in both groups (13% and 4.2% respectively) [34,35].

Conversely a meta-analysis by Sylvester et al., and Bohle and Bock showed reduced progression rate in favor of BCG compared to chemotherapy [36, 37]. These trials showed risk reduction in progression of disease only on maintenance BCG therapy.

For intermediate risk patients, intravesical chemotherapy is currently administered with the prophylactic intent of reducing recurrence rate. The risk of progression for this category is generally low and it is not taken as a primary end point. In the present study, on subgroup analysis, there was no significant difference in the recurrence rate between BCG and gemcitabine and this result is comparable to that of two meta-analyses focusing on intermediate risk group where there was no difference in recurrence rate between intravesical chemotherapy and immunotherapy in this risk group [38,39]. So, according to 2009 guidlines from European Association of Urology (EAU), the treatment of choise for intermediate risk group is either chemotherapy or immunotherapy and both types of treatment are accepted as standard in this subgroup [40].

In the present study, although, there was no significant difference in recurrence rate between BCG and gemcitabine in the intermediate risk group, there was a significant difference in the median recurrence free period in favor of BCG. This result is parallel to the finding that intravesical chemotherapy can clearly reduce the risk of recurrence of intermediate risk patients in the short term, however, in the long term, it has only a modest effect on the risk of recurrence [10, 33].

High risk group remains a challenge for the urologist. This study had shown a significantly lower recurrence rate with intravesical BCG compared to gemcitabine. However, there was no difference in progression rate or recurrence free survival between the two treatment groups. The result of our study is confirmed by a study of porena et al., where they evaluated the efficacy of BCG versus gemcitabine in high risk groups, the recurrence rate of BCG arm was significantly lower than that of gemcitabine (28.1 vs. 53.1) and there was no disease progression in both groups [41].

The recurrence rate of the high risk patients of BCG arm is comparable to that of Yumura et al., (21.1%), but higher than that of Gunlusoy et al., (6.1%) and lower than that of Brake et al., (11%), Kulkarni and Gupta (35%), Margel et al., (35%) Peyromoure et al., (42.1%),Lerner et al.,(50%),and Shahin et al.,(70%)[42,43,44,45,46,47,48,49].

In contrary to BCG, few studies have assessed the activity of intravesical gemcitabine in high risk patients. In phase II study of Bartoletti, et al., the recurrence rate of high risk patients treated with intravesical gemcitabine was higher than that of our study (77%) [24].

However, for high risk patients in whom BCG fails gemcitabine might represent a safe and effective option. In a study of Di Lorenzo comparing between gencitabine and BCG in those patients, recurrence rate was 52.6% vs. 87.5% for both groups respectively [50]. In another single arm study of Perdonà et al., the recurrence rate of the patients who received gemcitabine was 55% [51].

The progression rate of high risk patients of BCG arm is comparable to that of Yumura et al., (15.8%) but it is higher than that of Shahin et al., (3.3%), Kulkarni and Gupta, (12%) and Brake et al., (13%) and lower than that of,Margel et al., (18%),Gunlusoy et al.,(21.7%) and Peyromaure et al., (22.8%)[42,49,45,44,46,43,47].

The adverse events of the BCG were more marked than that of the gemcitabline arm. Intravesical gemcitabine was generally well tolerated and the local toxicity was minimal and generally described as rapidly self resolving. The published reports confirm the good tolerability with minimal local and systemic toxicity of gemcitabine in contrary to BCG which have frequent local and systemic adverse effects [52,5].On the otherhand, chemotherapeutic agents, such as MMC and doxorubicin, despite the low probability of systemic side effects, can give rise to severe forms of chemical cystitis. The molecular weigh of gemcitabine, 299.66 Da, is less than that of currently used intravesical drugs, yet is high enough to make significant systemic absorption unlikely (in an intact bladder) whilst being low enough for improved penetration of the bladder mucosa. The safety of intravesical administration of up to 2000 mg gemcitabine in 50 ml saline is substantiated by the evidence of how little gemcitabine is actually absorbed into the systemic circulation [53].

Some peculiarities of the present study are to be pointed out. Although a significant initial CR was achieved in high risk group of patients receiving gemcitabine, the majority of patients experienced recurrence within 12 months. This is not unexpected given the mechanism of cytotoxicity for chemotherapy and the brief duration of therapy in this study where there was no maintenance treatment. Conversely, immunotherapy is felt to induce a host response, against the tumour. This may be a potential reason that BCG is superior to chemotherapy

approaches in general.

One consideration to improve durability of response to gemcitabine is to consider maintenance therapy, although the role of maintenance therapy for intravesical agents is controversial [54,55]. In a meta-analyses of 11 randomized trials, Huncharek, suggested that chemotherapy for 2 years had the greatest effect on decreasing the recurrence rates [56]. This would not be unexpected given the log-cell kill obtained with chemotherapy agents, provided that chemoresistance does not develop.

A second consideration is that multiple-agent chemotherapy may be more successful than just using a single agent. Single agent chemotherapy is extremely limited in curing systemic disease, and the best results have been achieved with multiple agents. However, using two different chemotherapeutic agents, taking advantage of their synergistic effect, has not been attempted in intravesical approaches [15].In vitro studies have demonstrated a marked synergism between gemcitabine and MMc, the most commonly used intravesical chemotherapeutic agent [57]. Intravesical docetaxel was well tolerated in phase I trial, making a combination of docetaxel and gemcitabine a viable option [58,15].Several studies have shown that the efficacy of intravesical therapy can be increased by sequential administration of BCG and chemotherapy as MMc and gemcitabine. Intravesical chemotherapy and BCG have different mechanisms of action and may thus have a potentiating anti-tumour effect [59,60,5].

So, the early significant CR with intravesical gemcitabine and the high recurrence rate warrants investigating the role of maintenance therapy in high risk group in the future trials. Because of the low and transient adverse events and excellent cytotoxic effect of gemcitabine, more intense treatment schedules are now being contemplated [61].

Conclusion:

Gemcitabine is active and well tolerated for intravesical instillation.It is considered to be an efficient treatment for intermediate risk NMIBC. However, for high- risk group, it is inferior to BCG, but owing to its favorable toxicity profile, it may be useful for patients intolerant to BCG. Further studies are necessary to accumulate a larger amount of consistent data.

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Effect of a Rehabilitation Program on the Knowledge, Physical and Psychosocial Functions of Patients with Burns

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Abstract: Burns injuries may be severe enough to restrict daily activities in the home, school, and workplace. The nurse has a crucial role in implementing strategies for relevant effective care of patients with burn. The aim of the study was to evaluate the effect of a rehabilitation program on the knowledge, physical and psychosocial functions of patients with burn. This quasi-experimental study was conducted at the burn units of the Main University and Emergency Hospital in El-Mansoura on a convenience sample of 100 burn patients who were divided randomly into two equal groups: a study group for application of the intervention, and a control group to receive the routine management of the hospital. Data were collected using an interview questionnaire form and the Brief Burn Specific Health Scale (BSHS-B). A rehabilitation program with a booklet was developed after reviewing related literature to cover burn definitions, causes, types of burn, complications, proper diet, exercise, wound care, stress management. A booklet containing all the program materials was prepared in Arabic language. The program was provided to the study group patients, while the control group received the routine hospital's nursing management. The program effectiveness was evaluated through a posttest done for both groups. The study was implemented during the period from November 2006 to November 2007. Patients in the two groups had similar demographic as well as burn injuries characteristics. After implementation of a rehabilitation program, the knowledge scores were statistically significantly higher among study group patients in all areas as burns definition ($p<0.001$), treatment ($p=0.022$), exercise ($p<0.001$), and diet ($p< 0.001$). They also had significantly better scores of the Brief Burn Specific Health Scale (BSHS-B). The scores of knowledge and BSHS-B improved in both groups; however, the magnitude of the change was much lower in the control group compared to those in the study group. The study concludes that inclusion of the information needed by patients with burn in a rehabilitation program improves their knowledge, with consequent positive impact on their physical and psychosocial status. Therefore, the study recommends implementation of this rehabilitation program for all patients with burn injury. Nurses should be trained in patient education in order to be able to successfully implement such program.
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Key words: Burn, rehabilitation program, nursing

1. Introduction:

Burns are considered one of the most serious and devastating injuries among people of all ages. These injuries may be severe enough to restrict daily activities in the home, school, and workplace ***(Linton & Maebius, 2007; Park et al., 2008)***. Burn severity depends on its depth and the body surface affected. Burn care classified according to the depth of tissue destruction as superficial, partial thickness and full thickness injuries ***(Edelman, 2007)***.

Burn injury is associated with anatomical, physiological, as well as immunological alterations ***(Hosseii et al., 2007)***, which may end in infection ***(Church et al., 2006)***, and acute renal failure accompanied by a high death rate ***(Jaiswal et al., 2007)***. In addition to their dramatic physical effects, burn injuries frequently cause deleterious psychological complications ***(Mustonen and Vuola, 2008).*** These complications require comprehensive interdisciplinary rehabilitation focused on preventing long-term problems with scarring, contractures, and other problems that limit physical function, community integration, and return to work and other activities. Such program should begin during the acute treatment phase, and must be designed to meet each patient's specific needs ***(Heimbach et al., 2003).***

Nurses have an important role in the comprehensive interdisciplinary rehabilitation programs for burn survivors ***(Moi et al., 2008)***. Nursing interventions represent those activities that nurses do to assist the individual or family to move toward a desired outcome ***(Low, 2003).*** These interventions include the use of medications and non-pharmacological methods to achieve pain relief ***(Summer et al., 2007)***. They also include alleviation of the psychosocial and spiritual stressors through providing information and guidance, and improving the communication between nurse, physician and

patient ***(Kazak, 2005)***. Hence, the nurse is an important member of the rehabilitation team ***(Edgar and Bereton, 2004).***

Significance of the study

The researchers realized the importance of the problem of burn injuries through obtaining the statistics from the burn unit at Mansoura University Hospital in the year 2008. The number of cases who were burned accounted for 550 cases, i.e. almost two cases per day according to the Hospital Records in Mansoura University Hospital**.** Most of these patients had serious consequences that altered their biopsychosocial functioning. Given the crucial role of nurses in implementing strategies for relevant effective care of patients with burn, this study is intended to assess the utility of providing burn patients with information through a rehabilitation program in improving their knowledge as well as their physical and psychosocial functions.

Aim of the study

The aim of the study was to evaluate the effect of a rehabilitation program on the knowledge, physical and psychosocial functions of patients with burn. It was hypothesized that the implementation of this rehabilitation program will lead to significant improvements in knowledge, physical and psychosocial functions of patients with burn.

2. Subjects and Methods

The study was conducted at the burn units of the Main University and Emergency Hospital in El-Mansoura. A quasi-experimental design was used with a study and control groups, and pre-post assessment. The study participants consisted of a convenience sample of 100 burn patients. They were recruited according to the inclusion criteria of being adult with age 18-60 years, with partial or full-thickness burn regardless of the site, in the post-emergency stage (72 hours after the burn incident) who were willing to participate. The only exclusion criterion was having a chronic disease. These patients were divided randomly into two equal groups: a study group for application of the intervention, and a control group to receive the routine management of the hospital.

The data were collected using an interview questionnaire form developed by the researchers and the Brief Burn Specific Health Scale (BSHS-B). The interview questionnaire form included a section for patient's personal data as age, sex, and level of education, marital status, etc. The second section was for recording the data related to the burn injury as the causative agent, site, surface area, depth, etc. The third section was for pre-post assessment of patient's knowledge about burn injury nature (10 items), management (4 items), and recommended diet (8 items) and exercise (5 items). It was developed based on pertinent literature ***(Lewis et al., 2004; Perry and Potter, 2004; Slone, 2004)***. The total score was 49.

The Brief Burn Specific Health Scale (BSHS-B) was adopted from ***Kildal et al.(2001)***. It was used to assess the physical and psychosocial status of burn patients. It measures four domains in the area of physical status (simple ability, hand function, heat sensitivity, and treatment regimens), and five domains in the area of psychosocial status (affective, body image, interpersonal relationship, sexuality, and work). The scale consists of 40 items on a 5-point Likert scale ranging from 0 (extremely) to 4 (not/none at all). Thus, the total score ranges between zero and 160. A higher score indicates better physical and psychosocial status. This tool was also used for pre-post program evaluation.

Content validity:

The tools were reviewed by a panel of seven experts from medical-surgical nursing faculty and plastic surgery to ascertain their face and content validity and relevance.

Pilot study

A pilot study will be carried out on 10% of the patents under study to test the applicability, clarity and efficacy of the tools, then the tool will be modified according to the results of pilot study.

Rehabilitation program

This program was intended to cover the areas of knowledge and psycho-social disorders that burn patients may face with the aim of improving their health status. The content of program was developed after reviewing related literature ***(Ignativicius and Workman, 2002; Rochet and Zaoui, 2002; Ahmed,2003; Civaia et al., 2003; Timby and Smith, 2003)***. The program contents covered the areas of burn definitions, causes, types of burn, complications, proper diet, exercise, wound care, stress management. A booklet containing all the program materials was prepared in Arabic language.

Fieldwork

To carry out the study, the necessary approval was obtained from the director of Mansoura Hospital and the head of the burn department. The aim of the study and the procedures were explained to them to obtain their cooperation for data collection.

The study was implemented during the period from November 2006 to November 2007.

Patients were recruited according to the eligibility criteria. The researchers approached each patient individually at the post emergency stage after stability of the condition, explained to him/her the purpose and procedures of the study, and invited him/her to participate. Those who agreed were interviewed using the two data collection tools, and then assigned either to the study or the control groups.

A rehabilitation nursing program was provided to the study group patients, while the control group received the routine hospital's nursing management. The program consisted to seven sessions over two weeks (3 practical and 4 theoretical). Each session lasted 30-45 minutes. The first two sessions were designed to equip subjects with the necessary basic information related to the burn injury; one session was about diet, and three sessions about wound care and physical therapy, and the last session was about coping. The total time for the program was six hours. The training was four days per week in teaching sessions organized during the morning shift after patients receiving their medication and care, or after the visiting hours. Patients were handed the program booklet, with some explanations from the researchers regarding its use.
The researchers collected the data during the morning and afternoon shifts, four days per week. The personal interview took about 30 to 40 minutes. At the end of the program, its effectiveness was evaluated through a posttest done for both groups, using the same data collection tools.

Human rights

The researchers approached patients individually at the burn unit, explaining the purpose of the study, and the importance of rehabilitation in burn injury. Patients who were willing to participate were included in the study after obtaining their written consent, after informing them about their rights to refuse or withdraw at any time. Confidentiality of all information was secured. The study maneuvers could not entail any harmful effects on participants. Professional help was provided to all participants whenever needed.

Statistical analysis

Data entry and statistical analysis were done using SPSS 16.0 statistical software package. Quantitative continuous data were compared using Student t-test in case of comparisons between two groups. Qualitative categorical variables were compared using chi-square test. Whenever the expected values in one or more of the cells in a 2x2 tables was less than 5, Fisher exact test was used instead. In larger than 2x2 cross-tables, no test could be applied whenever the expected value in 10% or more of the cells was less than 5. Statistical significance was considered at p-value <0.05.

3. Results

Table 1 shows a similar distribution of various socio-demographic characteristics of patients in the study and control groups. About two thirds of them were less than 35 years age, with slightly more males in both groups (54.0%). More than one-third of the patients in the study (38.0%) and control (36.0%) groups had secondary education. Slightly less than half of the patients in the study group were married (46.0%), compared to 50.0% of the control group. As for job status, only about one third of the study (32.0%) and control (38.0%) group patients were working. None of these differences were statistically significant.

Table 2 provides the characteristics of burn injuries among patients in the two groups. About two-thirds of the burns were open flame, 64.0% and 62.0% in the study and control groups, respectively. Most patients had multiple site burns, with almost all of them having burns of their extremities. As for the extent of the burn, about half of the patients in the study (48.0%) and control (50.0%) groups had less than 21% surface area burn. However, the depth was mostly partial to full thickness in both groups. As the table indicates, no statistically significant differences were revealed between study and control groups.

Comparison of the pre-intervention knowledge scores of patients in the study and control groups revealed statistically significant differences (Table 3). It is evident that patients in the control group had significantly higher knowledge about treatment (p=0.006). After implementation of the intervention, the knowledge scores were statistically significantly higher among study group patients, compared to the control group patients in all areas of knowledge as burns (p<0.001), treatment (p=0.022), exercise (p <0.001), and diet (p < 0.001).

Concerning burn patient's physical and psychosocial status as assessed by the Brief Burn Specific Health Scale (BSHS-B), Table 4 indicates some statistically significant differences between the two groups before the rehabilitation program. These were related to body image (p <0.001), personal relationships (p =0.025), and sexuality (p <0.001). In all these differences, the scores were higher among patients in the study group. After implementation of the rehabilitation program, the table points to statistically significant differences between the two groups in almost all domains. The only exceptions were related to simple abilities (p =1.00), and affect (p =0.40). In all these differences, the scores were higher among patients in the study group.

Figure 1 illustrates the total changes in knowledge and BSHS-B scores among patients in the study and control groups at the pre and post-tests. It shows statistically significant improvements in study group patients scores of knowledge from 18.8 to 29.7 ($p<0.001$), and of BSHS-B from 77.3 to 110.0 ($p<0.001$). On the other hand, in the control group, the knowledge scores rose from 17.1 to 19.6, and the BSHS-B scores from 73.3 to 88.7. Although these improvements were also statistically significant in the control group, the magnitude of the change was much lower compared to those in the study group.

Table 1: Demographic characteristics of patients in the study and control groups

	Study (n=50)		Control (n=50)		X^2 test	p-value
	No.	%	No.	%		
Age (years):						
<25 years	21	42.0	18	36.0		
25-34 years	10	20.0	15	30.0		
35-45 years	10	20.0	12	24.0		
> 45	9	18.0	5	10.0	2.56	0.47
Sex:						
Male	27	54.0	27	54.0		
Female	23	46.0	23	46.0	0.0	1.00
Education:						
Illiterate	7	14.0	9	18.0		
Read & write	11	22.0	9	18.0		
Primary	8	16.0	6	12.0		
Secondary	19	38.0	18	36.0		
University	5	10.0	8	16.0	1.46	0.83
Marital status:						
Married	23	46.0	25	50.0		
Unmarried	27	54.0	25	50.0	0.16	0.69
Job:						
Not working	34	68.0	31	62.0		
Working	16	32.0	19	38.0	0.40	0.53

Table 2: Characteristics of burns among patients in the study and control groups

	Study (n=50)		Control (n=50)		X^2 test	p-value
	No.	%	No.	%		
Cause of burn:						
Flame	32	64.0	31	62.0		
Scald	17	34.0	13	26.0		
Electrical	1	2.0	6	12.0	--	--
Site:(@)						
Face and neck	19	38.0	26	52.0	1.98	0.16
Extremities	49	98.0	50	100.0	Fisher	1.00
Trunk	8	16.0	13	26.0	1.51	0.22
Surface area (%)						
15-	24	48.0	25	50.0		
21-	16	32.0	15	30.0		
26-30	10	20.0	10	20.0	0.05	0.97
Depth:						
Superficial	12	24.0	18	36.0		
Partial/full thickness	38	76.0	32	64.0	1.71	0.19

(@) Not mutually exclusive (--) Test result not valid

Table 3: Pre-post scores of knowledge about burns among patients in the study and control groups

	Knowledge score (mean±SD)		Student t-Test	p-value
	Study (n=50)	Control (n=50)		
Pre test:				
Burn	8.2±2.0	7.6±2.2	1.43	0.157
Treatment	2.2±0.0	2.4±0.5	2.83	0.006*
Exercise	1.0±0.4	1.0±0.4	0.0	1.00
Diet	6.8±1.9	6.2±1.6	1.71	0.091
Post test:				
Burn	12.6 ±3.0	8.1±1.4	9.61	<0.001*
Treatment	3.1±0.6	2.6±1.4	2.32	0.022*
Exercise	4.0±1.7	1.2±1.1	9.78	<0.001*
Diet	10.0±1.2	7.9±1.3	8.39	<0.001*

(*) Statistically significant at p<0.05

Table 4: Pre-post Brief Burn Specific Health Status (BSHS-B) scores among patients in the study and control groups

	BSHS-B score (mean±SD)		Student t-Test	p-value
	Study (n=50)	Control (n=50)		
Pre-test:				
Simple abilities	3.1±1.8	3.1±1.8	0.0	1.00
Hand function	6.6±7.6	6.6±7.6	0.0	1.00
Heat sensitivity	8.1±5.5	8.1±5.5	0.0	1.00
Treatment regimen	7.7±3.0	7.7±3.0	0.0	1.00
Affect	12.3±2.8	12.3±2.8	0.0	1.00
Body image	7.4±1.6	5.4±1.6	6.25	<0.001*
Personal relationships	10.7±2.2	9.7±2.2	2.27	0.025*
Sexuality	11.2±1.8	8.2±1.8	8.33	<0.001*
Work	7.7±3.0	7.0±3.0	1.17	0.246
Total	77.3±16.4	73.3±16.4	1.22	0.226
Post-test:				
Simple abilities	5.8 ±2.0	5.8 ±1.5	0.0	1.00
Hand function	11.8 ±6.5	9.4 ±4.9	2.09	0.04*
Heat sensitivity	11.7 ±4.2	8.5 ±2.8	4.48	<0.001*
Treatment regimen	11.9 ±2.3	9.0 ±2.4	6.17	<0.001*
Affect	14.1 ±2.0	13.7 ±2.7	0.84	0.40
Body image	8.6 ±1.6	5.4 ±1.4	10.64	<0.001*
Personal relationships	14.1 ±1.8	9.8 ±1.9	11.62	<0.001*
Sexuality	10.9 ±1.5	9.6 ±1.1	4.94	<0.001*
Work	10.1 ±3.1	7.6 ±2.1	4.72	<0.001*
Total	110.0±14.2	88.7 ±11.3	8.30	<0.001*

(*) Statistically significant at p<0.05

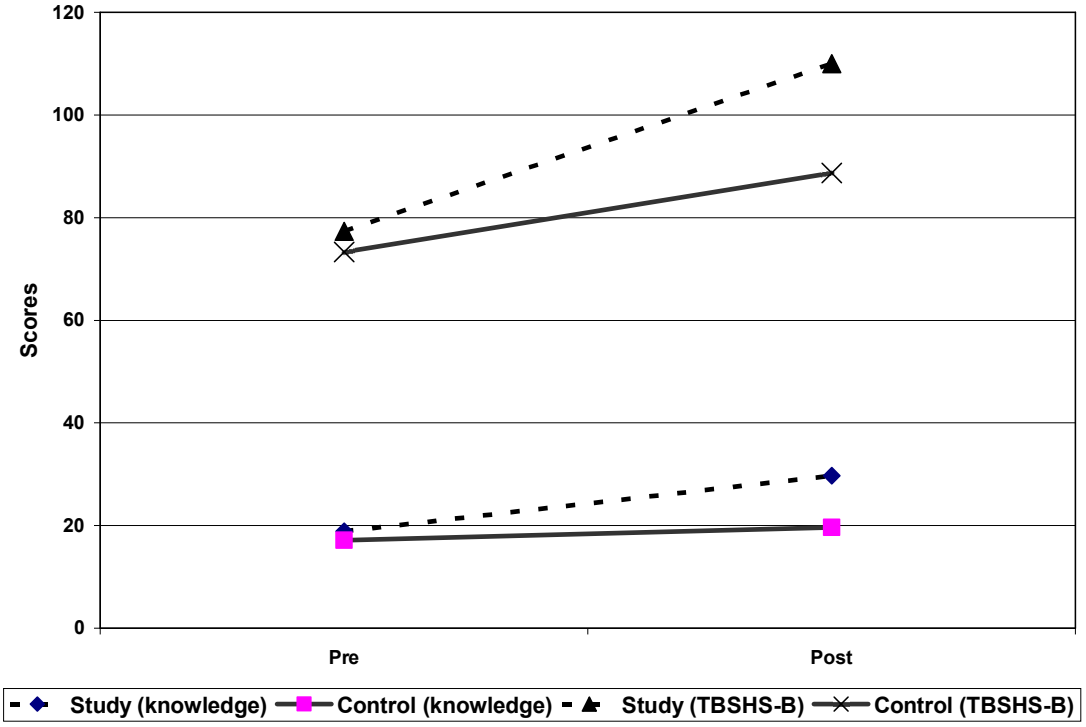

Figure 1: Pre-post total knowledge and Brief Burn Specific Health Status (BSHS-B) scores among patients in the study and control groups

Experts' opinions regarding burn knowledge assessment tool (No. of experts=7).

Items	Agree	
	Frequency	Percent
The used tool seems to assess patient's knowledge about burn injury	7	100.0
The words used in this tool are:		
Clear-	7	100.0
Correct-	7	100.0
Scientific-	6	85.7
Suitable-	7	100.0
Relevant	6	85.7
This tool serves the aim to assess patient's knowledge about burn injury	7	100.0
This tool can be used to determine patient's knowledge about burn injury	6	85.7
This tool is free from repetition among questions.	7	100.0
Parts of the tool reflect the pre-post assessment patient's knowledge about burn injury regarding the following:		
-Knowledge about burn injury natural (10 items).	7	100.0
-Management (4 items).	7	100.0
-Recommended diet (8 items).	7	100.0
Exercises (5 items).	7	100.0
The measure used is suitable and simple enough to determine nurse's knowledge about burn injury.	7	100.0

The great majority of the experts have agreed upon all items of the assessment tool and their percentages ranged between (85.7%) to (100%).

4. Discussion

This study was carried out to test the hypothesis that the implementation of a nursing rehabilitation program for patients with burn would lead to significant improvements in their knowledge, and their physical and psychosocial functioning. The study findings led to acceptance of the research hypothesis, as statistically significant improvements were demonstrated among patients in the study group after application of the rehabilitation program.

In order to show the effectiveness of the intervention, a control group similar to the study group patients was recruited, and the two groups were similar in all their demographic characteristics. There were slightly more males in the two groups. This might be attributed to occupational and recreational exposures among them, which is in congruence with ***Khan and Malik (2006)*** who reported a higher percentage of males among burn patients. Also, a high percentage of the patients were in the youngest age groups, below 35 years. This might be explained by more activity among this age group, which may predispose them to such incidents as also mentioned by ***Edlich et al.(2008)***.

In addition to similarity of the patients' socio-demographic data in the study and control groups, they also had the same burn characteristics. In both groups, most of the burns were due to open flame. This has been reported to be the most commonly encountered cause of burns in previous studies ***(Mabrouk et al., 2000; Maghsoudi et al., 2006)***.

Concerning the characteristics of the burn injury, most patients in both groups had multiple site burns, with a surface less than 20%. This is the most commonly encountered percentage of total body surface area for burns reported in burn centers ***(Willebrand et al., 2004)***. However, although the extent of the burns was mostly of moderate as classified by ***Schilling et al. (2004)***, the depth was mostly partial to full thickness. Nonetheless, no statistically significant differences were seen between study and control groups.

Concerning patients' knowledge about burns, the present study results revealed that the scores of patients in the study and control groups were generally low, with patients in the control group having better knowledge about treatment. However, after implementation of the rehabilitation program, the scores of patients in the study group demonstrated significant improvements, compared to the control group in all areas. The findings indicate success of the rehabilitation program, which is in agreement with the results of ***Belar (2000)*** and ***Mandal (2007)*** who reported similar improvements in the knowledge of patients with burn after implementation of educational programs.

The improvement in the knowledge of patients with burn in the study group was also associated with improvements in the scores of their Brief Burn Specific Health Scale (BSHS-B), which reflects improvements in their physical and psychosocial status. This again proves success of the rehabilitation program, which might be attributed to the content and process of the intervention, with more emphasis on patients' needs particularly those related to disturbed body image, personal relationships, and sexuality. The findings are in congruence with those of ***Mohamed (2003)*** who implemented a similar rehabilitation program at El- Minia General Hospital, and reported positive impact on patients' psychosocial status and physical functioning. Similar results were also reported by ***Sliwa et al. (2005)***.

The study findings revealed similar increasing trends between pre and post-tests on knowledge and BSHS-B scores. This points to an association between patients' knowledge and their physical and psychosocial status. The finding might be explained by the effect of having clear information on patient's psychological status, which would lead to more speedy and complete recovery. These results are in congruence with ***Baker et al. (2007)*** and ***Okhovatiana and Zoubine (2007)*** who demonstrated improved outcomes of patients with burn following rehabilitation programs that addressed patients' needs of information and psychological support.

The total scores of knowledge and BSHS-B demonstrated statistically significant improvements in both the study and control group patients after implementation of the rehabilitation program. However, the improvements were more obvious among patients in the study group. The improvements among control group patients might be attributed to contamination bias as some of these patients might have had access to the rehabilitation program materials as all the patients were in the same burn unit. This bias could not be avoided in the study setting, and might be considered as a limitation of the current study.

Conclusion and Recommendations

The study findings lead to the conclusion that burn patients are in need for information about burn injury and its management. Inclusion of such information in their rehabilitation program improves their knowledge, with consequent positive impact on their physical and psychosocial status.

Therefore, the study recommends implementation of this rehabilitation program for all patients with burn injury through the collaboration of various rehabilitation team members. Nurses should be trained in patient education and counseling in order to be able to successfully implement such program.

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Detection of Genetic Variation Among Three Isolates of *R. leguminosarum* Using Protein, Isozyme and DNA Fingerprints

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Abstract: Molecular markers, protein and DNA fingerprints were used to analyze the genetic variations among three *Rhizobium leguminosarum*. The frequency of genetic variability was detected in three isolates of *R. leguminosarum*. Variation in protein contents (89.25, 92.27 and 90.75 mg/g), protein banding pattern (10, 12 and 11 polypeptide bands) was observed for the three isolates of *R. leguminosarum* M, IS and F, respectively, with 40% polymorphic bands. The variation in peroxidase isozymes was 4, 6 and 5 bands, respectively, with polymorphic bands of 37.5%. Variation in each of protein banding pattern and peroxidase isozymes among the three isolates suggest that DNA fingerprint analysis could be used to show rapid and precise information about genetic variability. Ten arbitrary base primers were successfully used to amplify DNA fragments from three isolates. Three arbitrary 10 base primers (OP07, OP12 and OP18) revealed characteristic fragments where as 20% polymorphic fragments related to total amplified DNA fragments. Monomorphic 48% common amplified fragments as well as unique (genetic markers) 32% for the three *R. leguminosarum* isolates. The frequency of genetic variability (polypeptide, isozymes and DNA) was detected among 3 isolates of *R. leguminosarum* dependent somaclonal variation.
[Mohamed, H.F., Nor El Din, T.A., Abdel-Shakour E.H. and El-Dougdoug K.A. Detection of Genetic Variation Among Three Isolates of *R. leguminosarum* Using Protein, Isozyme and DNA Fingerprints. Journal of American Science 2011;7(8):435-440]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: *R. leguminosarum*; Peroxides isozyme (PRX); SDS-PAGE; RAPD- PCR; Genetic markers

1. Introduction

Modern developments is molecular biology have exploited the use of polypeptide fractions, isozymes and DNA fingerprints for genetic variability detection and represent on active area of research in microorganisms (Williamson, 1991). In Egypt, species of *Rhizobium* spp. are generally regarded as biofertilizers for legume crops; they attack causing considerable increasing of yield and quality of the produce. The impact of the *Rhizobia* on a crop and the relationship between the *Rhizobium* and host differ between *Rhizobium* species.

Most of published studies on detection of genetic variability and gene mutations were concentrated in the variations in chromosomes (genomes), isozyme polymorphism and biochemical diversity. A single set of arbitrary sequence 10 mers may be used for fingerprinting any species. The many advantages of RAPD markers over RFLPs or isozymes and protein markers accelerated the adoption of RAPD technology for the construction of genetic maps and fingerprinting (Tingey and Del Tuto, 1993). Wöstemeyer and Kreibich (2002); Sharma (2003); Swelim (2005); Aiat (2006) and Shash (2008) recoded that, the quality of DNA markers as RAPD PCR in detection genetic variability is well established for many microorganism.

Therefore, this study was conducted to employ SDS-PAG, DISC-PAGE and PAPD-PCR analyses as simple tools to use the molecular markers for the detection of genetic variability among *R. leguminosarum* isolates. Such marker(s) could also be used to assess the genetic relationships of three isolates *R. leguminosarum*.

2. Material and Methods

2.1. *Rhizobium leguminosarum* isolates

Three *R. leguminosarum* isolates namely M, IS and F were grown on yeast extract mannitol broth medium (YEM). These isolates were selected from fourty root nodules of faba bean plants from different locations (Amer, 2008a; b).

2.2. Determination of protein

Protein content was determined according to Bradford (1976) using bovine serum albumin as a standard.

2.2.1 Protein analysis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed as

described by Laemmli (1970) under reducing condition in discontinuous electrode buffer system. Protein samples were denatured and reduced completely by mixing the protein with in equal vol. of 2X sample buffer (Lucius *et al*, 1987) and the mixture was heated in a water bath at 92°C for 5 min and chilled on ice buffer. Treated proteins were centrifuged at 12000 rpm for 10 min. Electrophoresis was carried out at room temperature at a constant current at 25 mA for 1 hr. followed by 30 mA for 4 hrs. At the end of run, the gel was stained with Coomassie Brilliant Blue R-250 and destained in the stain solvent then photographed.

2.3. Peroxidase (PRX) isozymes electrophoresis

Electrophoresis of isozymes was performed according to Stegemann *et al.* (1986) and applied for three isolates of *R. leguminosarum* using PRX enzyme staining system. Peroxidase revealed the most variables with good-determined banding patterns.

2.4. Isolation of genomic DNA

Extraction of total DNA was performed according to the method of Wulff *et al.* (2002).

2.5. DNA amplification

Random amplified polymorphic DNA (RAPD) analysis was applied according to Williams *et al.* (1990) using 10 mers of five random oligonucleotide primers obtained from Metabion International AG. Lena-Christ-Str., Martinsried Deutschland as shown in table (1). Amplification was performed in 30 µl of reaction mixture containing 2.0 µl template DNA (25 mg, 2.0 µl *Taq* DNA polymerase (unit), 3.0 µl DNTPs (25 mol of each dATP, dCTP, dGTP and dTTP), 3.0 µl $MgCl_2$ (25mM), 3.0 µl PCR buffer (10X), 2.0 µl random primer (10 p mole) (Table 1) and 15.8 µl deionized water. The mixture was assembled on ice, overlaid with a drop of mineral oil. The amplification was carried out in DNA thermal cycle (MWG-Biotech Primuse) programmed as follows: Once cycle at 94°C for 4 min, and then 40 cycles; each cycle at 94°C for 30 sec (denaturation); 35°C for 1 min (annealing) and 72°C for 2 min (extension). One cycle at 72°C for 2 min then 4°C.

2.6. Gel electrophoresis analysis

All electrophoresis were carried out using a Pharmacia (GN-100) submorine gel electrophoresis apparatus. Agarose gel 1% was prepared in 1X TBE buffer (90 mM Tris-borate, 2 mM EDTA, pH 8.3). Total sample volume of 10 µl (5 µl of RAPD-PCR product, 4 µl dH_2O and 1 µl 6X loading dye) of each was loaded in gel wells and electorophoressed for 1.5 hr. The gel was stained with ethidium bromide solution (0.5 µg/ml) for around 10-15 min. DNA was visualized on a UV transillminator (1-254 nm) and photographed.

Table 1. Oligonucleotide sequences of the random primers.

Infinitive primer	Nucleotide sequences 5' → 3'
OPB-05	TGCGCCCTTC
OPB-07	GGTGACGCAG
OPB-10	CTGCTGGGAC
OPB-12	CCTTGACGCA
OPB-18	CCACAGCAGT
OPT-20	GACCAATGCC

3. Results

Variability among three *R. leguminosarum* in somaclonal variation should independent of bacterial genetics under environment to stress. Protein, isozymes and DNA were differed quantity and quality. Whereas, amino acid sequence of polypeptides are dependent on nucleotide sequence of their coding genes. Protein, isozymes and genomic DNA analysis detect the genetic variability among isolates of *R. leguminosarum*.

3.1. Protein banding patterns

The Results recorded in table (2) showed the differences in protein content, protein fractions and density, whereas, IS isolate showed to have the highest value of protein content followed by F isolate and M isolate of *R. leguminosarum*. SDS-PAGE profile of protein extracted from the three *R. leguminosarum* isolates was presented in table (2) and Fig. (1). It showed a total of 33 bands with different molecular weight values ranged from 200 to 8 KDa.

The protein bands of the three isolates varied in number and density of bands, whereas, M, IS and F isolates showed a total 10, 12 and 11 protein bands, respectively. The electrophoretic banding pattern of three *R. leguminosarum* isolates showed either absence or presence of different polypeptides in these three isolates. Six bands were polymorphic. They had the molecular weights of 150, 100, 89, 70, 40 and 8 KDa. Other bands were for only one isolate, such as 60, 30 and 12 KDa for F, IS and M isolates, respectively. Some of these bands appeared in each of the three isolates such as 200, 120, 50, 25, 20 and 15 KDa which were considered monomorphic ones. The presence of variability (polymorphic) among *R. leguminosarum* isolates was 40% in relation to the total number of bands.

3.2. Peroxidase iszoymes

Results of peroxidase isozymes are shown in table (3) and Fig. (1). The total number of peroxidase iszoymes shown in all three *R. leguminosarum* isolates were 8 isozyme bands. The number of these isozyme bands were 4, 6 and 5 for the isolates M, IS and F, respectively. The variability analysis of three isolates displayed a total of 8 bands. Of the eight isozyme bands, 3 bands of them were polymorphic, 2 others were common bands (monomorphic) over three isolates and 3 were unique bands. The percent of variability among 3 *R. leguminosarum* isolates was 37.5% relation to total peroxidase iszoymes.

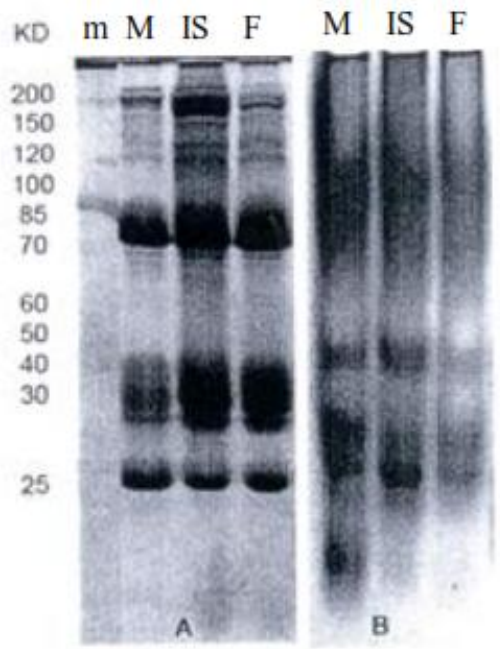

Figure 1. Illustrate the protein analysis and isozymes,

A. SDS-PAGE 12% of protein patterns extracted from 3- *Rhizobium* isolates.

B. DISC-PAGE 14% of polypeptides peroxidase isozymes of 3- *Rhizobium* isolates.

m = Marker.

M, IS, F = *Rhizobium* isolates.

3.3. RAPD-PCR analysis

The genetic diversity among the three *R. leguminosarum* isolates was evaluated, using six random primers (OPB-05, OPB-07, OPB-10, OPB-12, OPB-18 and OPT-20). Since, three primers (OPB-07, OPB-12 and OPB-18) gave genetic diversity among isolates (Tables 4 and 5). In total, 25 bands were produced, 5 of which were polymorphic. Primers OPB-12 and OPB-18 produced the highest polymorphic bands. The percentage of polymorphic bands ranged from 8.3% to 33.3% with an average of 20%. Number of monomorphic bands range from 1 to 8 with average 4 per primer. Number of unique bands ranged from 1 to 4 with an average of 2.7 per primer, it was observed that most of unique bands scored at F isolate.

Table 2. Protein content and fractions of *R. leguminosarum* isolates.

***R. leguminosarum* isolates**				**Polymorphism**
MW	**M**	**IS**	**F**	
KDa	**Density**	**Density**	**Density**	
200	++	++	++	Monomorphic
150	+	-	+	Polymorphic
120	++	+++	++	Monomorphic
100	-	++	+	Polymorphic
89	+	+	-	Polymorphic
70	+	+	-	Polymorphic
60	-	-	+	Unique
50	++++	++++	++++	Monomorphic
40	-	+	+	Polymorphic
30	-	+	-	Unique
25	++	++	++	Monomorphic
20	++++	++++	++++	Monomorphic
15	+++	+++	+++	Monomorphic
12	+	-	-	Unique
8	-	++	++	Polymorphic
10		12	11	No of polypeptides
89.25		92.27	90.75	Protein content (mg/g)

MW= Molecular weight markers (KDa).
Density band (–) = absent band, (+) = weak, (++) = moderate, (+++) = strong, (++++) = very strong.

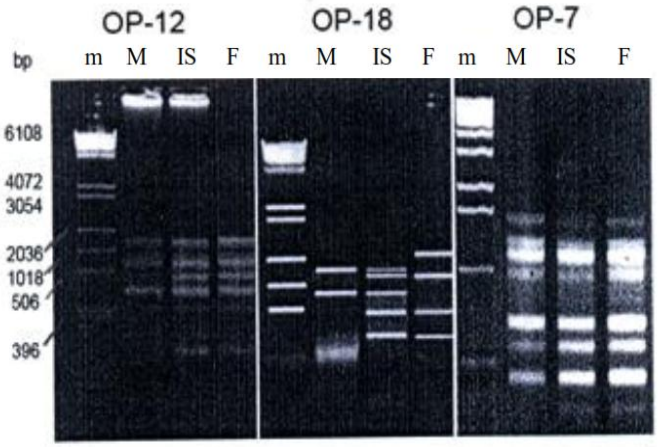

Figure 2. 1.5% agarose gel electrophoresis showing the DNA amplified PCR products of 3 *R. leguminosarum* isolates using 3 random primers.
m = Molecular weight DNA marker.
M, IS, F = *R. leguminosarum* isolates.

Table 3. Peroxidase (PRX) isozyme analyses of variable bands of three *R. leguminosarum* isolates.

No. of band	*R. leguminosarum* isolates			Polymorphism
	M	IS	F	
1	++	+++	+++	Monomorphic
2	-	+	-	Unique
3	++	+++	+	Monomorphic
4	+	+	-	Polymorphic
5	-	++	++	Polymorphic
6	-	++	++	Polymorphic
7	++	-	-	Unique
8	-	-	++	Unique
No of Polypeptides	4	6	5	

Table 4. RAPD amplified bands, polymorphic and genetic markers for *R. leguminosarum* isolates using random primers.

Random primers												
	OPB-07				OPB-12				OPB-18			
R. leguminosarum isolates												
	F	IS	M	Polymorphism	F	IS	M	Polymorphism	F	IS	M	Polymorphism
3000	++	-	++	Polymorphic	-	-	-	-	-	-	-	-
2960	++	++	++	Monomorphic	-	-	-	-	-	-	-	-
2504	++	++	++	Monomorphic	-	-	-	-	-	-	-	-
2296	+++	+++	+++	Monomorphic	-	-	-	-	-	-	-	-
2125	+++	++	++	Monomorphic	-	-	-	-	-	-	-	-
1625	+	+	+	Monomorphic	-	++	+	Polymorphic	++	+	++	Monomorphic
1560	-	-	+	Unique	-	-	-	-	+	+	-	Polymorphic
905	+++	+++	+++	Monomorphic	+++	-	-	Unique	++	+	++	Monomorphic
712	+++	+++	++	Monomorphic	-	++	+	Polymorphic	-	++	+	Polymorphic
510	-	-	-	-	-	+	-	Unique	-	-	-	-
413	+++	+++	++	Monomorphic	++	++	+	Monomorphic	+	++	+	Monomorphic
367	-	-	+	Unique	+	-	-	Unique	-	-	-	-
250	+	-	-	Unique	++	-	-	Unique	+	-	-	Unique
Total No. of fragments	10	8	11		4	4	3		5	5	4	

Table 5. List of primers, No. of bands and polymorphism in each primer.

Primer	No. of Polymorphic bands	No. of Monomorphic bands	No. of Unique bands	Total No. of bands	Polymorphism (%)	Monomorphism (%)	Uniqueness (%)
OPB-07	1	8	3	12	8.3	66.7	25
OPB-12	2	1	4	7	28.6	14.3	57.1
OPB-18	2	3	1	6	33.3	50	16.7
Total bands scored	5	12	8	25	20	48	32

4. Discussion

Protein patterns, peroxides isozymes and DNA fingerprints were used for analysis of somaclonal variations among three *R. leguminosarum* isolates namely M, IS and F (Amer, 2008a; b). They should be independent of *Rhizobium* genetics under environment stress. Whereas, amino acid sequence of polypeptides are depend on nucleotide sequence of their coding genes. Therefore, on electrophoretic analysis of protein and isozymes of three isolates of *R. leguminosarum* approximates the analysis of their genetic variation. The variation of three isolates was

detected via determination of polypeptides by using SDS-PAGE and peroxidase isozymes by using DISC-PAGE. Data presented in table (2) indicated the variation in both the number and density of protein fractions among the three isolates. Also, these results indicated the variation of protein content among the three isolates. This suggests that despite increasing the biodegradation of protein content, new protein types were detected due to the differential response of the three isolates to the effects of the environment stress. The same observation was reported by Swelim, 2005; Aiat (2006); Girgis *et al*. (2008) and Shash (2008). The syntheses of new proteins are due to the interaction between bacteria and environment conditions.

The enzymatic plots and their metabolic pathway are the most important factors affecting ecology conditions. The results showed that the levels of peroxidase among the three isolates were different. The increase in isozymes number plays an important role in the defense mechanism. Also, the increase in the enzyme activity has been detected by conditions in different microorganisms and ecology (Hammerschmidt *et al*., 1982).

DNA prepared from three isolates was found crucial for RAPD-PCR. The yields of DNA were determined by spectrophotometrically as 1.8, 1.9 and 1.75 µg/0.5 g cells of the three isolates M, IS and F, respectively. The PCR conditions for DNA analysis were optimized by investigating each factor individually. The optimized conditions were detailed in materials and methods section. A total of amplified DNA fragments raining in size of 3000 to 250 bp were observed using the three primers and were expressed as polymorphic with 5 bands (20%) monomorphic with twelve bands (48%) and 8 genetic markers (unique) with 32% which were detected among the three isolates. Interesting to note that, the three isolates were significantly varied.

The results of the present study gave preliminary informative DNA-based makers for 3 *Rhizobium* isolates. Also, optimizations of experimental conditions of PCR amplification are a prerequisite for the performance of RAPD analysis. This increased the reproducibility and efficiency of RAPD as a molecular marker technique. The three *Rhizobium* isolates were isolated from faba bean nodules (Amer 2008a; b). However, three random primers gave producible and very stable results peculiar to the same specific from different accessions. The other primers sometimes did not give the exact fingerprints for the 3 *Rhizobium* isolates. During the past years, numerous publications demonstrated the utility of RAPD markers for the analysis of the genetic variability among isolates of bacteria, fungi, actinomycetes and plant populations (perret and Broughton, 1998; El-Dougdoug *et al*., 2007; Swelim, 2005; Girgis *et al*. (2008) and Shash (2008).

The molecular mechanism underlying somaclonal variations have been attributed to chromosome breakage, single base changes in copy number of repeated sequences and alteration in DNA methylation patterns (Kaeppler and Philips, 1993 and Munthali *et al.,* 1996). The polymorphism in amplified products may be either a result from changes in the sequence of the primer binding site (e.g. point mutations) or changes which alter the size or prevent the successful amplification of the target DNA (e.g. insertions, deletions, inversions) as suggested by Rani *et al*. (1995).

It could be concluded that protein, isozymes and DNA fingerprint can be successfully used to detect somaclonal variations among the three *R. leguminosarum* isolates. Numerous researches proved that the sensitivity at protein, isozymes, and DNA analysis were sufficient enough to detect genetic variability in many of bacteria, fungi and actinomycetes (perret and Broughton, 1998; Sharma, 2003 Swelim, 2005; Girgis *et al*. (2008) and Shash (2008).

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3/5/2011

Geochemical Systematic Exploration of Stream Sediments in Shurchah Area (SE Zahedan)

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Abstract: Shurchah area is located 60 kilometers to the southeast of Zahedan. Zahedan granitoides and dioritic dykes have intruded in flysches of Eocene age. According to factors such as stratigraphy, lithology, tectonics and topological gravity of drainage patterns, 82 stream sediment samples have been taken from streams. Samples were analyzed using ICP-MS and AAS analytical methods. For eight elements Au, Ag, Cu, Pb, Zn, As, Sb and Hg factors such as error, frequency distribution, amount of sensored, background, threshold, anomaly, mean, mode and standard deviation, calculated individually. Among these elements, Sb with an average value of 10 ppm has been considered as anomaly regarding the spatial situation of the anomaly; it was determined to be in the central part of the study area. In addition, strong positive correlation was observed between gold- arsenic and gold- antimony.
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Key words: frequency distribution, antimony, anomaly, positive correlation

1. Introduction

The primary exploration for stream sediments study carried out in such drainage basins under different climate especially with few rains. Each sample can include chemical component of its upstream. Covering of vast area, ease of sampling, preparation and exist of wide aureole distribution are the advantages of method.

High probable pollution, river geomorphology changes, data interpretation difficulty and their relation to anomaly source are the disadvantages of method. (Hasani Pak &sharafoddin 2002).

The environment and effective process of ore deposition can be determined by geochemical studies of stream sediments. (Yazdi 2003). The sedimentary basin of study area has been divided into small parts due to separating stream sediment related to probable mineralization and those are not related to such mineralization which caused weakening of the anomaly.

The geochemical explorations method for stream sediments has been selected due to significance of geochemical studies of stream sediment which can help to determine environment, effective process in mineral explorations, concentration, distribution of elements in sediments and also use of tracer elements for promising area (Hasani Pak &sharafoddin 2002).

In stream sediments study method, many of minerals especially sulphide types was unstable and they decomposed because of oxidation and other chemical reaction. Such fact takes an important place in distribution of more minerals. Transportation and distribution of elements depends on their mobility in geochemical environment.

According to above reasons concentration changes of some elements such as Au, Ag, Cu, Pb, Zn, As, Hg, Sb in sediments and also relation and distribution of different element in sediment distribution, trace elements identification, exploration and accessibility to expected area are the objectives of this research.

The study area located in East Iran Flysch zone (Nehbandan –Khash Zone) (Stocklin 1972). This study covers Zahedan Granite, its age and genesis identification (Camp & Griffis 1981) (sadeghiyan 2007) also has been studied the granite rocks of Zahedan.

The geological location and access roads to Shurchah area falls 60Km SE of Zahedan city (Longitude 60° 54́ 52E, Latitude 33″ 10́ 29°N), The region is hot and arid. The weather is tolerable and the days are mostly sunny, it rains with few stormy periods, each lasting for several days (Fig.1).

2. Study methods

2.1 Sampling and their analyses

The design of sampling grid of this research covers 82 samples for sefid sang sheet with scale 1:50000 and map No. (8148-2) which introduce us the gravity center method. Factors such as stratigraphy, lithology and tectonics controlled the degree of gravity centre. According to definition, gravity centre of drainages are streams in which topologically can divided the drainage basin in two equal parts.

If stream itself is not gravity centre, amount (outermost) of streams (with number 1) located in both side are equals therefore, by using gravity centre method design on the map some points with high sampling density should be available. These points are: intrusive mass and its contacts, fault surrounding area in their intersection, alteration zones and areas which are located in upper part of hypabyssal intrusive mass (Hasani Pak & sharafoddin 2002).

To provide optimize design of sampling, drainages in a basin should be numbering.

The numbering procedure follows as each division of grid stream attributed to digit one out of the total upstream drainage, therefore digit one is belong to outer drainage and any division that derived from digit one marked as digit two, with continue numbering procedure will reach to the main stream in the outlet of Basin. If numbering is correct the obtained digit number for main stream at the outlet of basin will be equal to the total outer drainages of basin. (Hasani Pak & sharafoddin 2002).

Then, the outer streams should be divided to such samples which will take from stream sediment.

The obtained answer explains the number of drainages that should be consider for one sample. Thus

M=N/n

Where: N = the outer drainage, n = the number of samples have to be taken (Hasani Pak & Sharafoddin 2001).

During sampling process after selecting a suitable position with the help of topography maps and GPS through middle of streams, we have been taken the newest stream sediment deposits so that the new deviated drainage not connected to main stream (Fig2).

In this research 500 gram stream sediment samples with -80 meshes have taken and pulverized to -200 meshes. Due to wideness of area and frequency of elements only eight important elements including (Au, Ag, Cu, Pb, Zn, As, Hg, Sb), has been selected for geochemical processing.

The main factors in analyze method are to determine the sensored limit.

Basically, sensor amount in an element caused disturbance of data analyzing. (Goncalves 1998). In Geochemical explorations the importance and use of numerical values, related to elements is on account of relative comparison of them to each other.

To find out anomaly values, numerical values for each element related to its background value obtained, in which these values should be less than anomaly. (wellmer1998). Thus the different elements background value assigned and the suitable analyzing method will be select.

In this research owning to sensored limit elements, Gold element has been analyzed by ICP-MS method and other elements by AAS method analyzed which there were no sensored data in these eight selective elements.

2.3 Analyzing Error

In order to analytical machines accuracy control usually out of 10-15 samples, one repetitive sample (about 10-15%) has been selected and sent with secret codes to laboratory. Repetitive samples for eight mentioned selective element with rate of 35% has been chosen. Table1 shows the primary and repetitive data results.

With a repetitious analysis of geochemical samples the accuracy process has been studied. At the first steps Microsoft Excel and Thompson diagram used for measuring accuracy consideration (Fig. 3 to 9).

These charts are logarithmic which mean of values in x-axis and different of values in y-axis has been shown. After plotting the values if 90 % of data are below equivalent line of 10% and 99% of data are below equivalent line of 1%, will have about 10% errors.

As we can see in the diagrams all the plotted points are below two curves which shows the errors are less than 10%. (Silver data are less than one impossible to plot in this diagram).

3. Discussion

3.1 Outlier Value Test

In statistical discussion such values are significant different to other values are known as outlier value. For calculating the above values subtraction of large number to one value before it method was used. As data are not so extended, therefore this simple correction gives a reasonable result. (Hasani Pak & Sharafoddin 2001).

For example antimony element with a value of 52262 ppm from 80 taken samples is unusual and should be replace with nearest small value means 38293for its correction.

Thus, the maximum Antimony grade decreases from 52262 ppm to 52089.5 ppm therefore outlier values of other elements also calculated as shown in Table 2.

$$\Delta = \frac{52262 - 38293}{81} = 172.45$$

3.2 Determination of background, threshold limit, anomaly values

Sometimes, exploration data are asymmetrical with positive skewness, it means samples with low value (i.e. low grad) are abundance as compare to samples with high values(high grad). Therefore data distributions have been transformed, symmetric, and will be close to normal distribution often by using

transformed logarithmic function method. (Hasani Pak & Sharafoddin 2001). In data transformation logarithm the base 10 selected and the transformed values was used to calculated mean, variance, and other statistical factors.

For calculation and determination of background, threshold, anomaly values there is variety of methods which supposed all the data are normal. These values are obtained with the help of mean and standard deviation as follow: (Table.3).

<Mean + Sd = Background
Mean + 2Sd = Threshold limit
> Mean + 2Sd = Anomaly

3.3 Determining raw data stream sediment of elements

In geochemical data, the existence of abnormal value plays an importance role in selecting of promising area. Therefore, separation of anomal area and background is very important.

Exploration data in an ore deposit scale the Sturge's rule for estimating of class ranges used and has shown as follows:

$$\Delta = \frac{x_{\max} - x_{\min}}{1 + 3.322\log(n)}$$

Where:

(Xmax) and (Xmin) are the maximum and minimum values of data

N is No. of data

Due to determining raw data stream sediment of elements, statistic factors, frequency histograms and concentration distribute in statistic population for eight raw selective elements have been prepared in fig. 10 to 15.

As we can see from the above histograms all of them follow (L) distribution and maximum frequency is belongs to few values (Reimam 2005). In the samples, frequency distribution between low to middle values is dispersion, as in Cu element middle values shown more frequency.

The Au histogram more than 50 samples have less than 700 ppb values, 25 samples more than 1ppm and 5 samples have values near background or less than it. 74 samples had shown the anomaly grade.

The Antimony element, 13 samples are near threshold except for 2 samples from other samples (66 samples) are near anomaly. Stream sediment normal curve of antimony shows that middle frequency is about 300ppm. Frequency of AS element according to positive and strong correlation with Au is similar to gold frequency distribution curve and reasonably it can be antimony frequency and middle frequency of AS is related to about 35 ppm values.

3.4 Determining of correlation coefficient of elements

Owing to find out the relation between gold and its associated elements around mineralization and also to indentify process of different elements enrichment toward gold anomaly, we have chosen some of elements which their nature and frequency have been important in mineralization mechanism therefore their correlation coefficient calculated with gold element.

Spearman and Pearson methods were used for determining correlation coefficient between variables. Spearman method use when data frequency are not normal but for calculating of correlation coefficient through Pearson's method the data frequency should be normal (wellmer 1998). Elements correlation coefficient values of both spearman and Pearson's have been shown in Tables 5 & 6 respectively.

According to result of raw data, maximum correlation coefficient had shown between Gold and Antimony elements with 0.728 values and Arsenic with value of 0.641. In normal data maximum correlation is belong to Gold while Arsenic has a 0.995 value, it means Arsenic element is a good tracer for Gold and Antimony elements, as with the help of Antimony and Arsenic assemblies estimation can figure out reasonable Gold assembly. (Carranza 2008). figures16&17 indicated the regression curve of these elements.

3.4 Cluster Analysis

The linkage method used for data clustering on account of good connection of samples and variables as compare to return method. All cluster methods are base on resemblances matrix (Davis 2002). At the first step two samples or variables which are more similar to each other connected and at each repetition similar even cluster will connected together. (Fig. 18).

To identify paragenesis between eight selective elements in dendrogram cluster analysis of Shurchah samples, there is two important paragenesis groups as follow:

1. Zinc – Arsenic – Gold
2. Copper – Antimony – Mercury – Silver

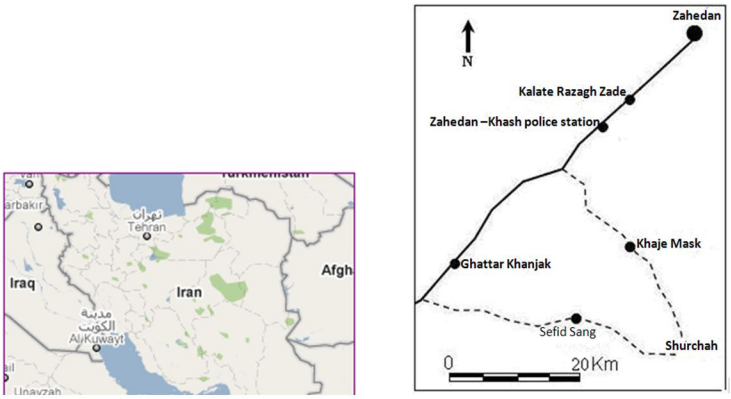

FIG.1. Geological location and the access roads to study area

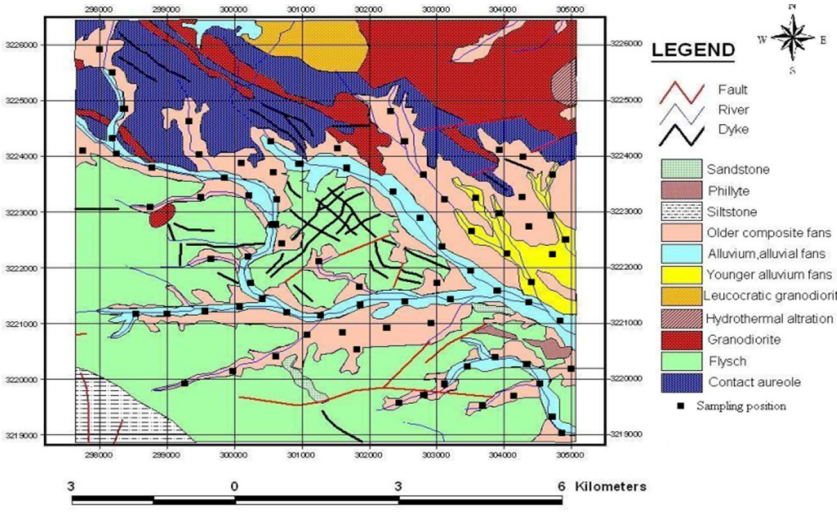

Table 1. Shows the primary (X1) and repetitive(X2) analyses results.

	Au (ppb)	Ag (ppm)	Cu (ppm)	Pb (ppm)	Zn (ppm)	As (ppm)	Sb (ppm)	Hg (ppm)
X_1	N27=13.3	N27=0.1	N27=29	N27=20	N27=288	N27=58	N27=2	N27=30
	N54=1332	N54=0.1	N54=4	N54=9	N54=6	N54=846	N54=2389	N54=15
	N81=0.5	N81=0.2	N81=10	N81=35	N81=5	N81=2	N81=3	N81=5
X_2	N27=12	N27=0.1	N27=28	N27=21	N27=287	N27=57	N27=3	N27=28
	N54=1335	N54=0.1	N54=3	N54=10	N54=6	N54=844	N54=2393	N54=15
	N81=0.5	N81=0.2	N81=9	N81=35	N81=5	N81=2	N81=4	N81=5

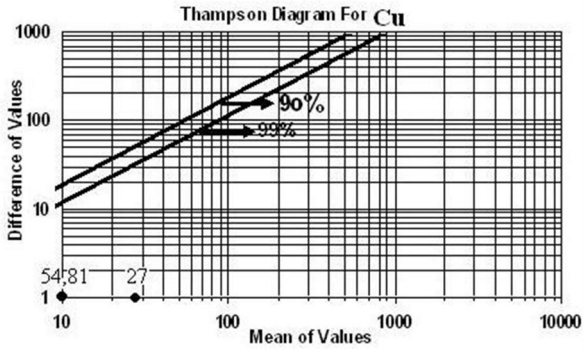

FIG.3. Shows Thompson diagram of Cu element

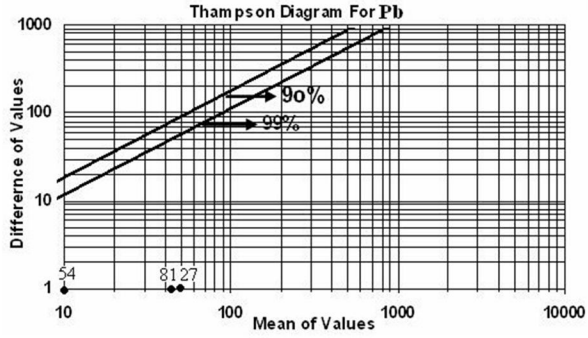

FIG.4. Shows Thompson diagram of Pb element

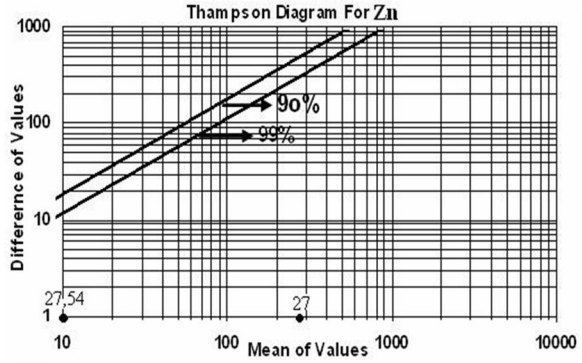

Figure5. ShowsThompson diagram of Zn element

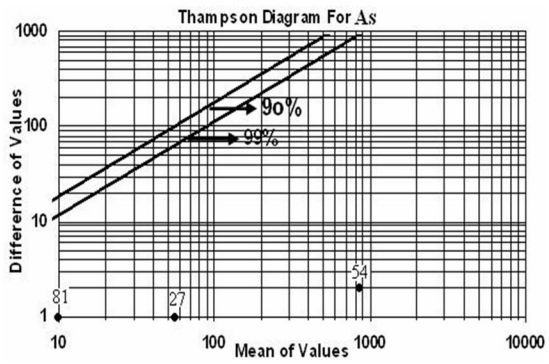

Figure6. Shows Thompson diagram of As element

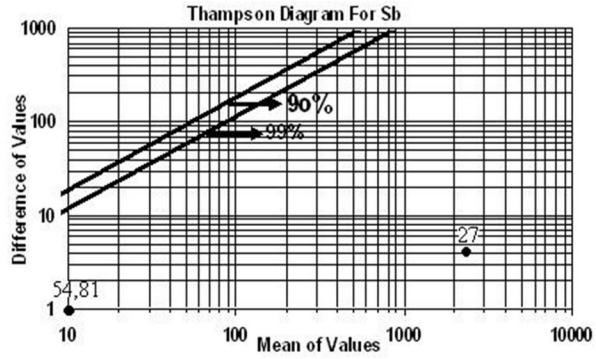

Figure7. Shows Thompson diagram of Sb element

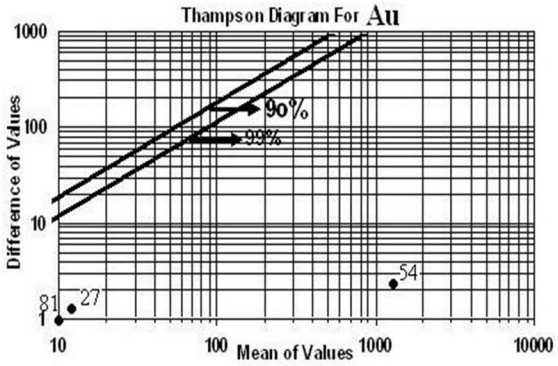

Figure8. Shows Thompson diagram of Au element

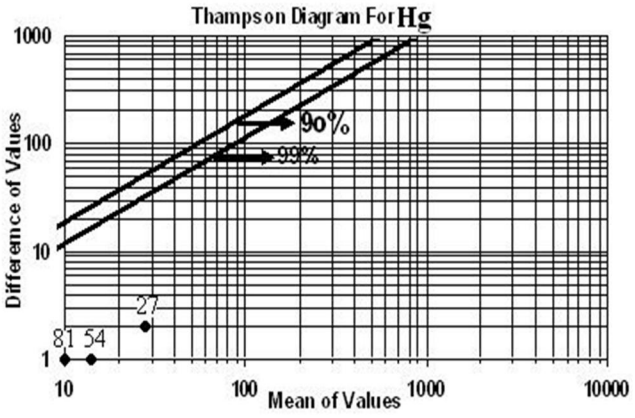

Figure9. Shows Thompson diagram of Hg element

Table2. Outlier Value and their correction

Element	Anomaly value	next Value	After of correction
Au	10567.5	7900.2	10534.5
Ag	274.2	149.9	272.7
Cu	14299	1770	14144.2
Pb	33500	23730	33379.4
Zn	80008	42709	79574.6
As	60430	51170	60315.7
Sb	52262	38293	52089.5
Hg	2250	1798	2244.5

Table.3 Determination of background, threshold, and anomaly limits of elements

Elements	Au (ppb)	Ag (ppm)	Cu (ppm)	Pb (ppm)	Zn (ppm)	As (ppm)	Sb (ppm)	Hg (ppm)
Background	<2.9	<18.53	<4.83	<9.1	<4.68	<12.7	<11.15	<5.08
Threshold	4.71	22.46	6.43	15.62	5.77	21.96	19.58	9.68
Anomaly	>4.71	>22.46	>6.43	>15.62	>5.77	>21.96	>19.58	>9.68

Table.4 Statistic factors calculated of raw data

Elements / Factors	Au	Ag	Cu	Pb	Zn	As	Sb	Hg
N	82	82	82	82	82	82	82	82
Mean	9.3171	2.9462	35.3854	70.2976	31.3263	26.0739	.3243	3.4110
Std. E. M	.66392	.21049	5.15960	7.45702	6.60107	1.09507	.02426	.53489
Median	8.0000	2.5600	20.0000	59.3000	20.0000	26.0000	.3000	2.0000
Mode	10.00	1.00	15.00	47.00	24.00	22.00	.20	1.00
Std. Dev.	6.01208	1.90609	4.67222E1	6.75262E1	5.97752E1	9.91630	.21964	4.84363
Variance	36.145	3.633	2.183E3	4.560E3	3.573E3	98.333	.048	23.461
Skewness	1.197	1.242	3.244	4.785	5.619	.172	1.880	3.903
Std. E. of Skw.	.266	.266	.266	.266	.266	.266	.266	.266
Kurtosis	1.219	1.776	11.651	22.815	32.743	-.640	5.495	20.572
Std. E. of Kurt.	.526	.526	.526	.526	.526	.526	.526	.526
Range	28.00	9.00	265.80	416.00	426.00	45.00	1.29	33.80
Minimum	2.00	1.00	2.00	27.00	7.00	8.00	.01	.50
Maximum	30.00	10.00	267.80	443.00	433.00	53.00	1.30	34.30
Sum	764.00	241.59	2901.60	5764.40	2568.76	2138.06	26.59	279.70

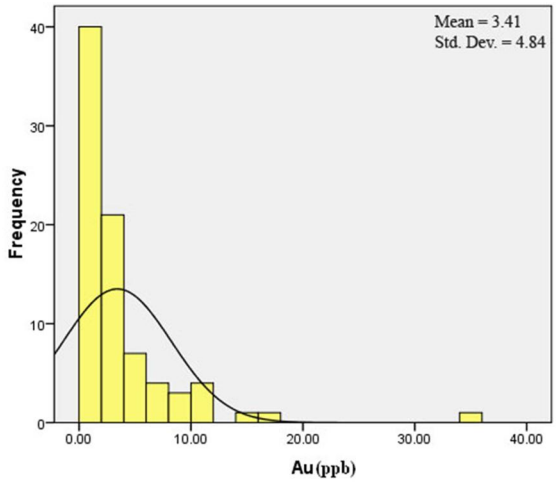

FIG.10. Frequency histogram of Au element

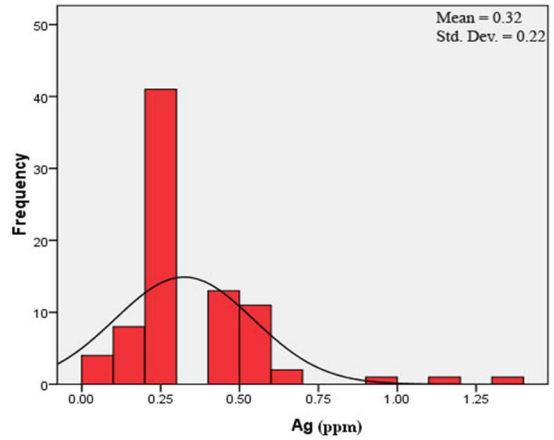

FIG.11. Frequency histogram of Ag element

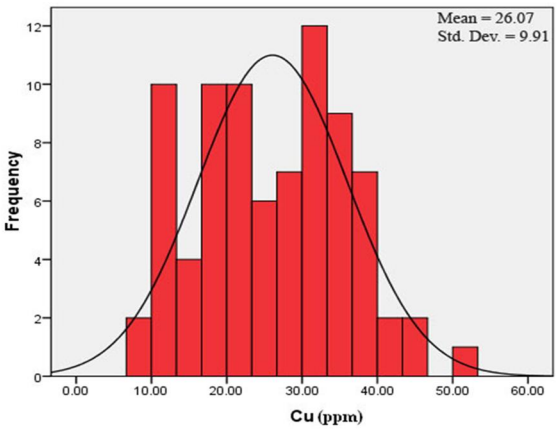

FIG.12. Frequency histogram of Cu element

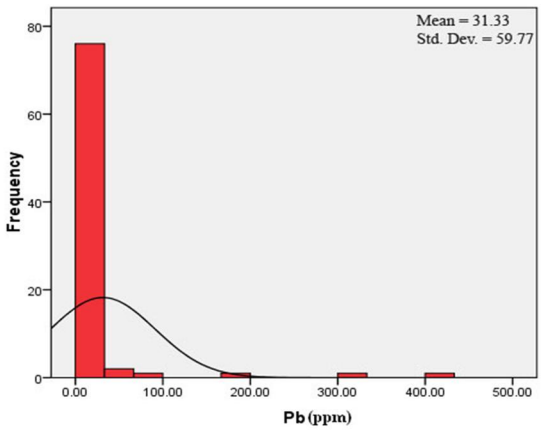

FIG.13. Frequency histogram of Pb element

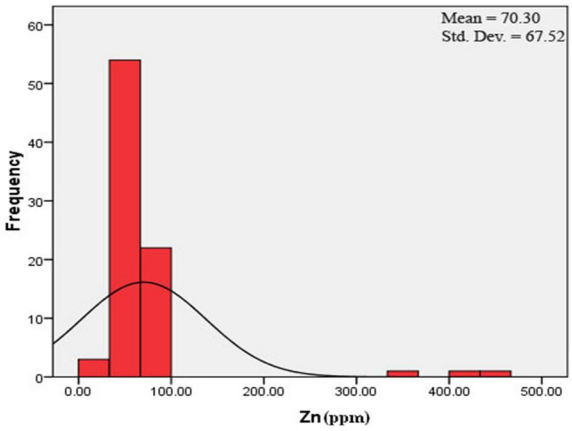

FIG.14. Frequency histogram of Zn element

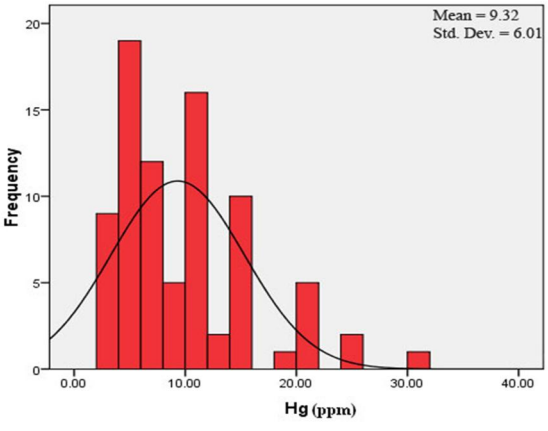

FIG.15. Frequency histogram of Hg element

Table6. Calculation of elements correlation coefficient using Pearson's method

Element	Au	Ag	Cu	Pb	Zn	As	Sb	Hg
Au	1	.018	-.036	.240*	-.027	.995**	-.004	.566**
Ag	.018	1	-.163	-.139	-.161	.007	.167	.058
Cu	-.036	-.163	1	.557**	.792**	-.033	-.034	-.020
Pb	.240*	-.139	.557**	1	.448**	.194	-.029	-.033
Zn	-.027	-.161	.792**	.448**	1	-.025	-.025	-.015
As	.995**	.007	-.033	.194	-.025	1	-.002	.570**
Sb	-.004	.167	-.034	-.029	-.025	-.002	1	.036
Hg	.566**	.058	-.020	-.033	-.015	.570**	.036	1

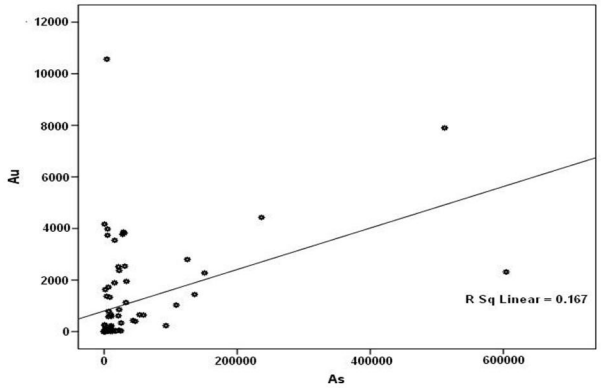

FIG.16. Shows dispersion of Gold with Arsenic elements along with regression curve

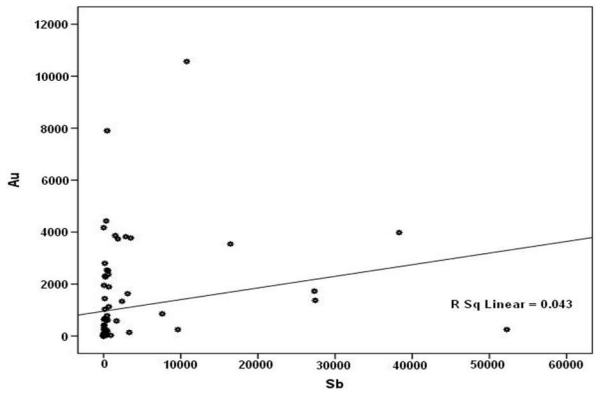

FIG.17. Shows dispersion of Gold with Antimony elements along with regression curve

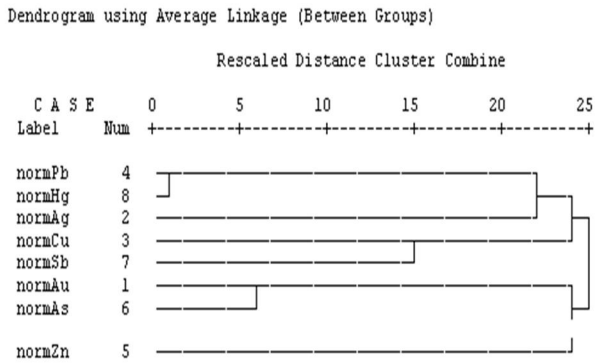

FIG.18. Shows Dendrogram related to stream sediments

Conclusion

1. In the raw data more correlation coefficient is a value of 0.827 between Gold and Antimony element and in normal data more correlation coefficient is a value of 0.995 between Gold and Arsenic.
2.Studies of elements correlation show in spite of strong and positive correlation between Gold, Arsenic and Antimony, Arsenic element can be introduce as Gold and antimony tracer and the estimation of Antimony and Arsenic piles can give reasonable information of Gold pile.
3.The elements such Copper, Silver and Mercury because of their strong chemical affinity with sulphide have fewer solvencies in water and in compare to other studied elements they are mostly in upstream.
4. Antimony element with a value of 10 ppm located in latitude 29° 17′ 46″ and longitude 61° 00′ 51″ has been selected as anomaly limit out of eight selective elements.
5. According to cluster analysis there are two important group as follow:
1. Zinc – Arsenic – Gold
2. Copper – Antimony – Mercury – Silver
Therefore chemical and paragenesis behavior elements of each group are similar.

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The effect of Justice in attraction of customer and for compensate of incomplete services (Case study: Hotels of Shiraz City)

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Abstract: Successful companies know that right now low price strategy could not provide any guarantee for market leading. Customer satisfaction with regard to high services and supportive strategies are important tools for pay attention to the requests of customers. It is a need for all companies to focus on customer. Although prevention is so much better than treatment, but receiving no claims is inevitable. Since providing incomplete services is inevitable but pay attention to this matter is so much important for service providers. The present paper intends to consider how much compensate of incomplete services would be effective on customer satisfaction for considering its claim and finally its support of company. Perhaps it is possible to apply it as a marketing tool as well. For this purpose we appointed relevant customers of Shiraz hotels and distributed a questionnaire in order to have the idea of 140 guests who had different claims to the chairman of the hotel. The result was positive effect of justice on prevention from negative consequences of incomplete services.
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Key words: Betterment of Services Process, Procedural justice, Interactional justice, Distributive justice

1. Introduction

All organizations are obliged to have a continuous attention to their customers' idea and provide required guidelines for better compliance with their orders. Considering the ideas and claims of customers is one of the methods for pay attention to their needs and requests. When we will understand it's important that witness of more loyalty of customers. Providing non-conditional obligation for following up justice is a fundamental item in each transaction. It is special for considering any claims which may arise after finding any defects in servicing. It is necessary for the customer to be ensured about suitable consideration of his claims. Of course nobody is effective on justice system. One of the fundamental characteristics of a responding system is to be justice. The customers who have received incomplete and non-successful services may request for compensating services and /or select other service providers and/or manage for other functions (Weun *et.al*, 2004).

According to different researches about betterment of services (It means different activities in which an organization may try to consider all received claims by customers who have been provided with incomplete services) show that there is different relations between conceptual justice and behavioral reactions. This is a sign of the effect of justice on psychology and behavioral ideals of the consumer.

One of the suitable goals of a servicing center is to provide complete services. But today customers have more expects than before and little royalty. Since they have different requests, therefore it is inevitable for service providers to furnish them with incomplete services. In fact the high level of services provided by an agency is not really important but what is important is lack of any mistakes in their daily contacts with customers. It is common in all fields of services and is basically due to some exclusive specifications of services such as simultaneous and/or indirect production & consumption.

Therefore most customers may experience relevant problems of services. In complete services may lead to non-satisfaction of customer and his /her further claims. As a result any lack of providing of services and /or weak presentation is major factor in losing customers. By the way how it is possible to solve this problem besides other services? Researchers believe that renovation and betterment of incomplete services are major factors in creation any relation with customers who had firstly no more satisfaction. It is impossible to prevent from customer's claims in Services Industry instead it is so much expensive to provide better management for responding to customers against incomplete services.

According to the researches we can say that customers may evaluate the reaction of service providers against incomplete services and efforts for compensating the services with a 3-dimensional justice including Distributive justice, Interactional

justice and Procedural justice (Blodgett et .al 1997); (Chebat & Slusarczyk, 2005); (Karatepe, 2006); (Smith *et. al* 1999); (Tax *et. al*, 1998). The 3-dimensional theory of justice is an important factor for providing any evaluation of situation by customers. It is famous for reaction of service providers against any claims for incomplete services (Kim *et. al*, 2009).

This paper intends to consider this matter that any understanding of claiming customer of justice processes for betterment of services will have great effects on his/her behavioral reactions and feelings.

2. Material and Methods

"Service Recovery Paradox" includes different efforts for compensation nay mistakes in providing of services. In 1992, McCollough & Behradavaj created this term for those customers who have received incomplete services. Then after receipt of compensating services, they find more satisfaction in comparison with those who had not received any incomplete and bad services (Kim et. al, 2009)

According to the researches we can say that customers may evaluate the reaction of service providers against incomplete services and efforts for compensating the services with a 3-dimensional justice including Distributive justice, Interactional justice and Procedural justice (Blodgett et .al 1997); (Chebat & Slusarczyk, 2005); (Karatepe, 2006); (Smith et. al 1999); (Tax et. al, 1998). Adam's theory (1963) about 3-dimensional condition of justice is a powerful tool for displaying any evaluation of customers about reactions of service providers against any claims of incomplete services. (Kim et. al, 2009).

Most of these researches focus that to be effective is different based upon the type of justice. In 1987, Westbrook noticed that satisfaction is obvious as a central item of behavior and after purchase of services. All pre- purchase beliefs are related to conceptual framework after it. Any satisfaction of providing better services has the role of an intermediate for making a relation between justice of behaviors and their attitudes after arising of claims (Tax et. al, 1998). Researches made by Karande et. al (2007), Karatepe (2006), OK et. al (2005), Mattila & Patterson (2004), McCollough et.al (2000), Smith et. al(1999), Tax et. al (1998), Smith & Bolton (1998), Blodgett et. al (1997) focus on this point that conceptual justice of customer in betterment of services process is so much effective on satisfaction of the mentioned betterment.

Confidence in services is a pre-requisite for further relations between customer and service supplier. This is because most customers have no chance only to purchase a produce without any previous experiences. If the customer is satisfied of services, it may lead to his/her confidence of service supplier. When confidence is formed that customer is ensured about integration and confidence situation of all services (Kim *et. al*, 2009). Any satisfaction out of services betterment may have further confidence as well.

According to the results of researches, any consideration of claims will have positive relation with customer royalty and return of customer. This may finally lead to long-term benefits (Tax *et. al*,1998). Effective process of services betterment means increasing the customer satisfaction and more probable return of customer. There is a relation between satisfaction and royalty of customer. There is a positive relation between word-of mouth advertisement and return of customer (Kim *et. al* 2009). There are a lot of common ideas about positive effect of word of mouth advertisement of satisfied customers while non-satisfied customers are the origin of negative oral advertisements (Wirtz & Chew, 2002).and also some researchers such as Amany et.al (2010), Dodangeh et.al (2010)and Mazandarani (2010) did statistical works in this field.

Confidence and obligation are effective factors in any transaction and on final decision of both parties. Confidence has a positive effect on the motivation of useful relation between the buyer and seller. Morgan & Hunt (1994) provided a marketing relation model in which we have confidence as a key variant for creation of ong-term relation with a customer. Mental advantages of confidence are more important than special behavior or social priority in any relation between customer and the organization. As a result any confidence of customer to the supplier of services is a reason for its relation. A service supplier (like hotel) may apply the confidence as the most powerful marketing tool for increasing the royalty of customers. It is special when suppliers of services tries to have more confidence and reduce any further claims in future.

Services

Service is a complex term. It has different meanings and includes a wide scope from personal services up to a special product. This term includes a wider scope as follows:

- Service; is an activity and/or benefit provided by one party to another which is basically non-specified and without ownership of nothing. The result would be physical and/or non-materialistic. (Kotler & Armstrang ,2000)
- Service is a result requested by customers (Harvey, 1998)

- Service is providing more benefits which is basically intangible and/or an exclusive product or important element which may meet any needs of customer in an intermediate form (Palmer & Cole, 1995).
- Service is a process including one set of more or less intangible activities in mutual transactions naturally and not necessarily between customers and personnel and/or physical resources and /or products or service providers for providing a solution for the customers (Gronroos, 2000).

Incomplete services

A non-accessible goal is providing incomplete services like a host's services for all customers. Since business in hotel has some special characteristics, any incomplete services may cause non-satisfaction of customer while he/she may lead to a claim. Any reply from servicing agency to the customer's claim is unhappy experience with a sensitive and critical situation. A weak reply (from agency side) means the second time of disappointment by the consumer, while a betterment of mistakes may convince the agency that it is possible to attract the customer once more (Davidow, 2003).

Followings are different examples of incomplete services in hotel management:

There is a wrong key for guest's room. When the guest enters into the room, he/she finds it dirty, there is no handkerchief in the room, water of bath is cold, telephone is disconnected, he/she is waiting a lot for specifying the room number, T.V. is out of service, Cooling/heating system is out of service, Climatic situation of the room is so much hot / cold for the guest, Quality of foods is low and beds are dirty.

One of the following four reactions are natural when the guests of a hotel are non-satisfied of relevant services: (Kim et. al 2009).

1- They leave hotel in silence.
2- They tell bad things about the hotel.
3- They refer to persons in charge for claim.
4- They remain in hotel in spite of their non-satisfaction.

Claims system

A claim means a declaration of non-satisfaction about a product and/or services. Claims management means analysis, planning, performing and controlling all activities of a company facing with different claims. (Hosseini *et. al*, 2007).

If there is a settlement of disputes system, they may find more royalty against company in comparison with those who did not reveal their non-satisfaction. It is about %34 of customers with a major claim who has referred to the same company after settlement of his/her claim. Regarding the non-important cases it is about %52. In case of quick settlement of any claims about %52 (major claims) and %95 (little important) of customers will refer to the same company (Kotler, 2006)

According to the investigations those customers who may claim are always satisfied customers. It is seldom for satisfied customer to refer to another company in case of quick experience of a product and experiencing another problem as well. Claims of a real buyer are generally quick and resulted from a misunderstanding. Companies should try to handle customer's claims carefully. Customer satisfaction is a key factor in his/her intends to purchase the same product in future. Satisfied customers may speak about their suitable experiences with others and may have word of mouth advertisement. As a result there is a positive relation between satisfaction level of a customer and his/her royalty (Hsunhsu *et. al*, 2006).

It is possible to say that used systems and processes for optimization of claims handling are the best investment opportunities for a company to be considered at the time of service providing for customers. This is because in current economic conditions any finding and meeting the needs of customers is really hard. The most important duty is to create good and nice relationship with customers. Suitable consideration of claims and planning of marketing & betterment systems may cause an increase in sale rate of company and upgrading its public reputation. In addition there is a high rate of investment return in the field of good planning of marketing systems and betterment in comparison with other investments. In fact, claims are "Free of charge" information supplied by customers and may cause betterment of services quality (Horotiz, 2003). In spite of all above-mentioned advantages, only little number of companies intend to invest in the field of planning and providing suitable systems for considering any claims made by customers.

Justice

Previous researches about real meaning of justice were led to 2-dimensional meanings of this concept which are Distributive justice and interactional justice. Both attitudes mean independent dimensions of justice while by completion of researches, a 3-dimensional model was proposed under the titles of distributive justice, procedural justice and transactional justice for better understanding of individuals' conceptions about justice. Most of researches are focusing on all three

dimensions of justice (Charash & Spector, 2001), (Gilliland & Chan, 2001).

1-Procedural justice: It means a process for better understanding of required services for customer and real justice in different steps of procedure and processes for compensation of the incomplete services (Mattila, 2001). Procedural justice means customer's understanding of regular efforts for betterment of services such as "To be on-time", "Speed", "Reflection", "Processes control", "Correct executive policies", "Correct & suitable methods" (Blodgett *et. al*, 1997), (Chebat & Slusarczyk, 2005), (Maxham & Netemeyer, 2002), (Smith *et. al*, 1999), (Wirtz & Mattila, 2004).

2-Interactional justice: It means the quality of behavior of persons through the performance of processes and specifying justice judgments. Any descriptions and explanations are useful in better understanding of justice. It may increase any probable justice decision making procedures as well (Krag & Lind, 2002).

The real meaning of transactional justice is betterment of evaluation services and a degree of justice related to organizational personnel with customer in betterment process of services (Sparks & McColl-Kennedy, 2001). There are some special methods proposed for interactional justice and betterment of services such as : "Humility", "Respect", "Interest", "Careful listening", "Confidence", "Explanation", "Sincerity", "Apologize" and "Communications". (Blodgett *et. al*, 1997); (Mattila, 2001); (Smith *et. al*,1999), (Wirtz & Mattila, 2004).

3-Distributive justice: In this research we have distributive justice involved with presented results to customer at the time of betterment of services. Followings are different proposals in hotel management which may present to customer with following up distributive justice: "Providing gifts"; "Discount"; "Upgrading the room level"; "Free of charge room and/or foods and any types of services" and any types of other compliance and replacement services (Blodgett *et. al*, 1997); (Chebat & Slusarczyk, 2005); (Smith *et. al* 1999); (Wirtz & Mattila, 2004).

Word of Mouth advertisement (WOM): Arndtez (1967) explained word of mouth advertisement as follows:

It means a face-to-face relation between a receiver and a messenger in a way that receiver will receive different information about the name, address; type of product and/or services from non-trade channels (fig 1) (Ghaffari Ashtiani, 2006).

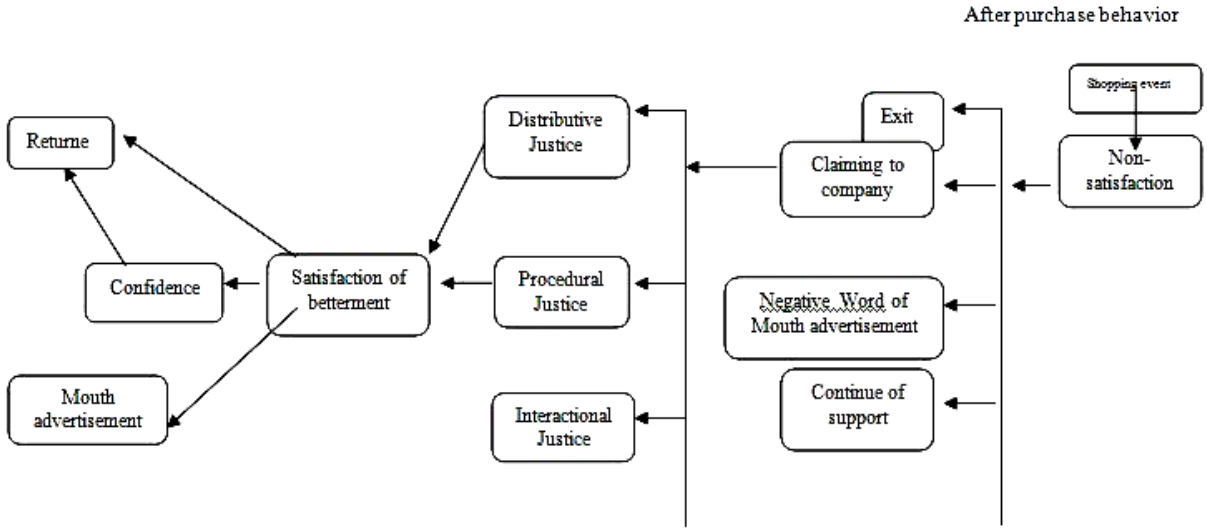

Fig1: Explanatory model of research

Theories of research

1st theory: Distributive justice has a positive relation with satisfaction of betterment.

2nd theory: Procedural justice has a positive relation with satisfaction of betterment.

3rd theory: Interactional justice has a positive relation with satisfaction of betterment.

4th theory: Satisfaction of betterment has a positive relation with confidence.

5th theory: Satisfaction of betterment has a positive relation with word of mouth advertisement.
6th theory: Satisfaction of betterment has a positive relation with return of customer.
7th theory: Confidence has a positive relation with word of mouth advertisement.
8th theory: Confidence has a positive relation with return of customer.

Research method

1-**Data collection**: Measuring tool in this research is questionnaire for further data collection. For this purpose standard questionnaire of Kim *et. al* (2009) is used accordingly. This questionnaire includes 21 questions based upon Likert 5 options scope. There are 7 parts for questionnaire questions and some questions for measuring of these specifications (research variants). Sample of this questionnaire is enclosed.

2-**Statistical population, sample & sampling method**: The statistical population of this research is "residing guests at Shiraz hotels. They have claimed with hotel owner about received services and requested for betterment of their services". Statistical population includes only Iranian guests with omission of foreign guests from it. This was because of a far distance of Iranian hotels with international standards for foreign guests. Perhaps there are a lot of defects in Iranian hotels, while Iranian guests evaluate the hotel according to their own knowledge of Iranian hotels.

Totally there are 20 hotels in Shiraz with different degrees (It means hotel and hotel-apartments, Motels and Guest rooms) which upon a lot of followings up only 10 hotels were ready to cooperate. All questionnaires placed at hotel for guests.

Following formula is for calculation of sample volume:

$$n = \frac{Z_{\alpha}^{2} pq}{d^{2}}$$

Therefore at 95-percent insurance level we have sample volume equal to 140. For this purpose we distributed about 180 questionnaires from which 40 questionnaires omitted due to the incomplete condition of analysis process and finally we performed our analysis on 140 questionnaires. Also sampling methods is available on sampling basis.

3- **Narration & Remaining situation of measuring tools**: After considering the subject literature and recognition and description of variants and effective factors of subject and benefiting from key essay and mentioned questions, we started to prepare primary draft of questionnaire. After discussing the case with guidance & consultant professors and interview with different managers of hotels with enough experiences in this regard, we prepared standard questionnaire required for data collection.

Chronbach α method was used for ensuring about remaining of measuring tools applied in this research. The amount of Chronbach α for this questionnaire was 0.9328 which is a sign of high level of validity.

4-**Data analysis method**: Explanatory statistic methods were used for data regulation in relevant tables of abundance distribution and calculation of centralized and diffused indexes. Pierson combination interval was applied for calculation of combination intervals between the variants accompanied with meaningful test for specifying its meaning. Also Multi-regression analysis was used for specifying the type of relation between variants by mathematical relations.

Data analysis: Klomogroph- Smirnoff test may approve the normal situation of statistical population of this research. For finding correct or incorrect condition of research theories we used Pierson Combination interval of meaningful test in which t statistics is used accordingly.(Table1)

Table 1

result	**Error**	**Sig** (Significant level)	supposition
The first theory is aaceptable	%5	000/0	first
The second theory is aaceptable	%5	000/0	second
The tird theory is aaceptable	%5	000/0	tird
The fourth theory is aaceptable	%5	000/0	fourth
The fifth theory is aaceptable	%5	000/0	fifth
The sixth theory is aaceptable	%5	000/0	sixth
The seventh theory is aaceptable	%5	000/0	seventh
The eight theory is aaceptable	%5	000/0	eight

Since the combination interval between interactional justice is greater than satisfaction of betterment, it is concluded that interactional justice has, in comparison with two other dimensions of justice, a more powerful relation with satisfaction of betterment and after that we have distributive justice, finally we have interactional justice with lower effects on satisfaction of betterment against two other dimensions of justice.(Table 3)

Table 3: Correlation coefficient between independent variable and satisfaction of betterment in order of importance of dependent variable .

Coefficient determination	Correlation coefficient	Dependent variable	Independent variable
0/349	0/591	Fair communication	satisfaction of betterment
0/184	0/429	Distributive Fair	
0/146	0/382	Procedural Fair	

Also it was revealed that there is a little powerful relation between confidence and word of mouth advertisement in comparison with any relation between satisfaction of betterment and word of mouth advertisement.(Table4)

Table 4: Correlation coefficient between independent variable and oral advertisements in order of importance of dependent variable .

Coefficient determination	Correlation coefficient	Dependent variable	Independent variable
0/582	0/763	confidence	Oral advertisements
0/526	0/725	satisfaction of betterment	

The relation between confidence and return is also a little powerful than any relation between satisfaction of betterment and return.(Table 5)

Table 5: Correlation coefficient between independent variable and oral advertisements in order of importance of dependent variable.

Coefficient determination	Correlation coefficient	Independent variable	Dependent variable
0/49	0/700	confidence	Return
0/482	0/694	satisfaction of betterment	

In case of careful look at all made tests, it is obvious that meaningful level in all eight theories is equal to 0.000. Therefore we claimed that all eight theories of the research would be confirmed even at mistake level of %1.

Multi regression analysis:

In this research we considered analysis model by the use of multi-lines regression analysis technique and by step-by-step method in SPSS software. Regarding this model, the considered variants for researcher is estimation of satisfaction from betterment, three variants of distributive justice, procedural justice and interactional justice. According to the results of regression analysis it is obvious that if we consider all three dependant variants it is better possible to estimate satisfaction of betterment. The **F** test may approve this regression.

The results of analysis through a software for testing the intervals of independent variants may specify that all three variants have a meaningful relation with dependent one (Because their meaningful level is smaller than mistaking level).(Table 6).

Table 6:Variable Coefficients that affect on satisfaction1

Variable	Coefficients	**ateB**	**t**	**giS**
fixed	2/464		2/252	0/026
Fair communication	0/437	0/484	6/650	0/000
Distributive Fair	0/251	0/201	2/824	0/005
Procedural Fair	0/251	0/175	2/399	0/018

Regarding the mentioned analytical model, both variants including satisfaction from betterment and confidence are considered variants of the researcher for estimation of word of mouth advertisement. Therefore by the use of data analysis it is obvious that both dependent variants such as confidence and satisfaction of betterment are better estimators of word of mouth advertisement against a situation in which we have estimated the word of mouth advertisement only with confidence. The **F** test will confirm meaningful condition of this regression.

The results of analysis through software may provide independent intervals with both independent variants with positive intervals. This means that any increase in their quantity may result an increase in word of mouth advertisement. Here regarding the greater confidence interval against satisfaction from betterment it is revealed that when we have both confidence and satisfaction with each other, confidence has a greater relation against satisfaction from betterment with word of mouth advertisement. (Table7)

Table 7: Variable Coefficients that affect on oral Advertisements

Variable	Coefficients	ateB	t	gis
fixed	-0/215		-0/429	0/669
confidence	0/450	0/571	7/318	0/000
satisfaction of betterment	0/176	0/283	3/622	0/000

With regard to analytical model, both considered variants of researcher for estimation of return are satisfaction from betterment and confidence. When we consider both dependent variants of confidence and satisfaction from betterment, we have better estimator for return against when we want to estimate only the return with confidence. The **F** test may approve this regression.

Furthermore the results of analysis through the software may provide different independent variants intervals in which we have confidence and satisfaction from betterment with positive intervals. It means that any increase in their quantity may cause further increase in their return. Here with regard to greater amount of confidence interval in comparison with satisfaction from betterment interval when both variants are present simultaneously, confidence has a greater relation with return in comparison with satisfaction from betterment. (Table 8)

Table 8:Variable Coefficients that affect on oral

Variable	Coefficients	ateB	t	gis
fixed	-0/450		-0/705	0/482
confidence	0/359	0/407	4/574	0/000
satisfaction of betterment	0/267	0/383	4/306	0/000

Regarding all presented steps in this research it is possible to claim that following up justice in betterment of service may have a positive effect on satisfaction from betterment. Also satisfaction from betterment may lead to customer support of the hotel.

4. Results and Discussions

The present research approves that different relations are governing on conceptual justice and behavioral reactions. This study shows that justice is so much effective on psychology and behavioral ideals of the consumer. The results of this research approve the positive effect of justice in betterment of services and further satisfaction from betterment, confidence and return of customer to the hotel.

Therefore justice in betterment of services is one of the most important factors for obtaining customer satisfaction.

Interactional justice would be more effective against distributive & interactional justice for satisfaction of betterment. This finding is the same as all obtained results from Blodgett *et. al* researches (1997) on different goods and services and also researches made by Tax *et. al* (1998) on different customers of insurance services, emergency, tourism tours and bank. Furthermore it has been also confirmed by any researches made by Davidow (2003) on customers of hotel and consumers of sale services through Post and Karatepe (2006) on guests of hotel.

The findings of the present research confirm that satisfaction from betterment has an intermediate role in further connection between justice of behaviors and attitudes after arising any claims. According to the results, it is revealed that satisfaction from betterment of service has a positive and important role in further confidence. Also the results show that if the guests are satisfied from betterment of services process, this may lead to a positive word of mouth advertisement and also re-election the same hotel for their residing as well. This may confirms that any efforts for betterment of services may increase any relation with customer (McCollough , 2000). This may cause a positive attitude in customer against the services and as a result a powerful relation between customer and company (Smith *et. al*, 1999), (Tax *et. al*, 1998).

Furthermore the created confidence may cause a positive attitude against the hotel and start to word of mouth advertisement for certainly re-selection of the same hotel once more.

The results of research show that claiming customers for whom there are faithful betterment of service would be changed into more powerful supporters than customers who have not receive any incomplete services.

Some proposals for hotel managers:

When we have a bad service, any effective reactions of service suppliers may even resulted in a powerful relation between customer and company and increase of royalty while providing short and incorrect replies means losing customer for second time and disappointing them from good service. Therefore it is certainly the failure of hotel in attraction of customer. Therefore it is necessary to have effective efforts for betterment of services after receipt of bad ones with an exact programming in order to result a long-term relation with customer.

The results of this research provide a useful attitude about any reactions against those guests who have

received incomplete services. Also there are some proposals for the hotel managers as follows:

It is necessary to have a quick start of betterment of services process for the mentioned guests

To consider final degree of interactional justice accompanied with a training program in which we may determine all behaviors of non-satisfied guests and suitable reaction of all personnel

To consider different types of damage compensation in financial form and provide them for all full-time & half-time personnel to present to all guests in order to have quick & suitable replies to bad situations. Hotel managers are obliged to teach their personnel to provide suitable proposal for compensation any damages to customer according to different levels of defects and guests situations.

To provide an educational program for creation suitable procedures and correct policies for increasing procedural justice against quick reaction of customer claims with a simple process and free from complex procedures within shortest possible period of time.

Managers are obliged to lead their personnel and provide learning chances and suitable education for them in order to enable them make better decisions at sensitive conditions. It is important for managers to have higher confidence in abilities of personnel in responding any claims. It is also better to prevent from minor management. This may increase the mood of personnel and more successful in obtaining satisfaction of customer. Hotel managers should allocate considerable allowance for those personnel who may have suitable behaviors in such conditions in order to enrich their voluntarily cooperation feeling.

It is better to appoint a person in charge of betterment of services process in all hotels with required authorities in order to have this process in its best situation against claiming customers..

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Bioaccumulation, Fate and Toxicity of Two Heavy Metals Common in Industrial Wastes in Two Aquatic Molluscs

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Abstract: Accumulation of chromium (Cr) and cadmium (Cd) by the bivalve *Caelatura (Caelatura) companyoi* and the gastropod snail *Cleopatra bulimoides* was determined. The fate of these metals through soft parts and shells of the molluscs was investigated. Toxicity studies of different concentrations of Cr and Cd on the survival of these organisms were performed. Results showed that both molluscs could accumulate both metals to a large extent and could tolerate their toxicity to high limits, as these metals were accumulated mainly in their soft parts, while small amounts were absorbed by their shells. It could be concluded that the investigated molluscs can be used as bioindicators for pollution of aquatic ecosystems by such heavy metals.
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Key words: Heavy metals, Bioaccumulation, Bivalves, Gastropod snails, Toxicity.

1. Introduction:

The term, trace metal, identifies a large group of metallic elements which are present in limited amounts in nature as well as in living organisms. Heavy metals are among the major concerns in waste water treatment (Salchi *et al.,* 2008). They are often derived from heavy industry, such as electroplating, battery factories and mining operations. The problems associated with trace metals contamination were highlighted in industry because of their large discharges and because chromium and cadmium are especially dangerous to aquatic organisms and can be bioaccumulated in the food chain (Medina *et al*., 1986; Brown and Louma, 1995; Pip, 1995). Also chromium and cadmium are metallic contaminants that have known essential function in human physiology (Barak and Mason, 1990). Moreover, fishes and bivalves are known to accumulate metals in their bodies .Since they constitute an important human food (Lopez-Artiguez *et al.,* 1989; El-Deek *et al.,* 1994; Schuhmacher and Domingo, 1996; Zyadah, 1996; Sidoumou *et al.*, 1997), they are potentially an indirect source of metals entering the human body, but they may also suffer from a wide range of metabolic, physiological and ecological factors. As the concentration of metal increases, the accumulation of metal and its damage effect increase (Cain and Louma, 1986; Buschiazzoa *et al.*, 2004). Cumulative effects of metals or chronic poisoning may occur as a result of long term exposure**.**

Reports on the occurrence and accumulation of heavy metals in bivalve molluscs have led to the concern about contamination of commercial mussel. They have generally been limited to salt water forms (Pentreath, 1973; Valiela *et al.,* 1974).

Hemelraad *et al.* (1986, 1987 and 1988) have published a series of papers concerned with the effect of cadmium on freshwater clams.

Hemelraad *et al.* (1990) studied the effects of cadmium on freshwater clams *Anodonta cygnea* and the interaction of Cd with the essential elements Na^{+}, K^{+}, Ca^{2+}, Hg^{2+}, Fe^{2+} and Zinc.

Abdel Moati and Farag (1991) studied the rate of bioaccumulation in Edku lake and El-Fayomy (1994) found that the marine clam *Cardium edula* in lake Manzala accumulated more heavy metals than the examined fishes.

The freshwater bivalve *Caelatura (Caelatura) companyoi* and the snail *Cleopatra bulimoides* were chosen for the present study as they are widely distributed along the River Nile from Assiut (Upper Egypt) to Damietta branches (Lower Egypt).

These species were chosen to determine their ability for the accumulation of Cr^{3+} and Cd^{2+} in their soft parts and to show the extent of their tolerance towards these pollutants in the freshwater ecosystems. It also aimed to examine the possibility of using these organisms as bioindicators for heavy metals pollution. This study might provide basic information for detecting the current status of heavy metal pollution in freshwater ecosystems.

2. Materials and Methods

Collection of the investigated mollusc samples

Samples of molluscs were collected from Ismailia canal at the industrial area of Abu-Zabal, El-Kalyubia Governorate during autumn 2008, in polyethylene bags filled with water from the same habitats and brought alive to the laboratory. Specimens were cleaned before being placed in glass aquaria (30x25x20 cm). They were left for two weeks in dechlorenated tap water that was changed twice per week.

From the same habitat, water samples were collected in polyethylene bottles, preserved by adding 2.0 ml of concentrated nitric acid and stored in a refrigerator till analysis. Bivalves and snails were labeled and frozen until analysis (FAO, 1983; UNEP, 1984).

Bioaccumulation and fate of the investigated heavy metals in the sampled molluscs

To study the bioaccumulation of each heavy metal, 60 specimens of each mollusc species were immersed in chromium and cadmium solutions of different concentrations. Twice per week, 3 specimens were taken randomly from each mollusc species for the analysis of the metal accumulation in their soft parts and shells.

Toxicity

Adult specimens of *C. companyoi* and *C. bulimoides* were divided into groups (10 each) to determine the LC_{50} of cadmium and chromium of different concentrations which kill 50% of the specimens in a certain time. Three replicates of each experiment were used.

The samples were then digested with nitric acid and perchloric acid (Saki *et al,* 1995) and finally diluted with double-distilled water. The digested solutions were analysed by Flame Atomic Absorption Spectrophotometer (Varian Spectra AA-300 plus). Data were expressed in mg/g weight and the concentration factor (C.F) values were calculated according to the equation:

$$C.F. = \frac{\text{Concentration of metal/g wet weight of animal}}{\text{Concentration of metal/g water}}$$

3. Results

Cadmium and chromium levels in field samples

Table (1) shows the concentrations of Cd and Cr in soft parts of the molluscs under investigation and in water from the field. The concentration factor of chromium was higher than that of cadmium.

Table 1: Concentration of chromium and cadmium in soft parts and shells of molluscs and water from the field.

Molluscs	Heavy metals	Conc. in Water (mg/l)	Conc. in Soft parts (mg/g wet wt)	C.F.	Conc. in Shells (mg/g wet wt)	C.F.
Caelatura (Caelatura) companyoi	Chromium (Cr)	0.022	1.520	69.0	0.20	9
	Cadmium (Cd)	0.012	0.576	48.0	0.084	7
Cleopatra bulimoides	Chromium (Cr)	0.022	1.100	50.0	0.13	6
	Cadmium (Cd)	0.012	0.504	42.0	0.06	5

C.F.: Concentration Factor

Bioaccumulation of cadmium and chromium in the investigated molluscs

Tables 2 – 5 and Figures 1-2 show the measured concentrations of Cr and Cd in the soft parts and shells of *C. companyoi* and *C. bulimoides*, as well as the concentration factor values. Results showed that cadmium is much more toxic than chromium. Therefore, the bioaccumulation studies were continued with a lower concentration of cadmium than that of chromium. The concentration factor values for chromium reached 325 and 278 for the investigated bivalve and snail, respectively after 20 days at 20 mg/l, while in the case of cadmium it increased to reach 88 and 72 for the bivalve and the snail at a low concentration of 3 mg/l. Higher concentrations of cadmium (10-20 mg/l) showed great toxicity.

Table 2: Mean bioaccumulation of chromium (20 mg/l) in the soft parts and shells of the bivalve *Caelatura (Caelatura) companyoi* as a function of time.

Immersion time (days)	1	2	5	10	15	20
Concentration in soft parts (mg/g)	3.4	8.4	16.8	28.2	42.4	65.0
Concentration factor (C.F.)	17	42	84	142	212	325
S D	0.095	0.261	0.144	0.227	0.178	0.180
Concentration in shells (mg/g)	1.6	1.6	2.2	2.2	2.0	2.2
Concentration factor (C.F.)	8	8	11	11	10	11
S D	0.325	0.298	0.413	0.206	0.342	0.200

Table 3: Mean bioaccumulation of chromium (20 mg/l) in the soft parts and shells of the gastropod snail *Cleopatra bulimoides* as a function of time.

Immersion time (days)	1	2	5	10	15	20
Concentration in soft parts (mg/g)	1.6	3.8	13.4	20.4	37.2	55.6
Concentration factor (C.F.)	8	19	67	102	186	278
S D	0.255	0.344	0.261	0.254	0.276	0.172
Concentration in shells (mg/g)	1.0	1.2	1.6	1.4	1.6	1.6
Concentration factor (C.F.)	5	6	8	7	8	8
S D	0.104	0.096	0.072	0.055	0.070	0.092

Table 4: Mean bioaccumulation of Cadmium (3 mg/l) in the soft parts and shells of the bivalve *Caelatura (Caelatura) companyoi* as a function of time.

Immersion time (days)	1	2	5	10	15	20
Concentration in soft parts (mg/g)	0.33	0.96	1.56	2.64	2.58	2.64
Concentration factor (C.F.)	11	32	52	188	86	88
S D	0.085	0.076	0.114	0.062	0.140	0.137
Concentration in shells (mg/g)	0.21	0.24	0.3	0.3	0.27	0.3
Concentration factor (C.F.)	7	8	10	10	9	10
S D	0.306	0.144	0.092	0.315	0.023	0.602

Table 5: Mean bioaccumulation of Cadmium (3 mg/l) in the soft parts and shells of the gastropod snail *Cleopatra bulimoides* as a function of time.

Immersion time (days)	1	2	5	10	15	20
Concentration in soft parts (mg/g)	0.27	0.84	1.44	2.16	2.19	2.16
Concentration factor (C.F.)	9	28	48	72	73	72
S D	0.115	0.179	0.380	0.605	0.378	0.713
Concentration in shells (mg/g)	0.15	0.15	0.18	0.18	0.21	0.21
Concentration factor (C.F.)	5	5	6	7	7	7
S D	0.323	0.356	0.405	0.793	1.117	1.503

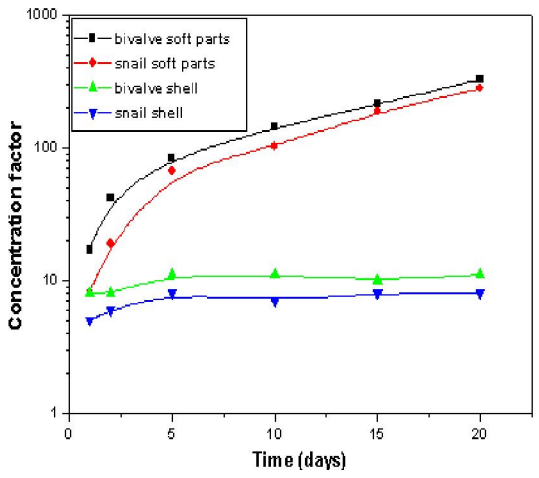

Fig.1: Mean concenteration factor of Chromium in soft parts and shell of the bivalve *Caelatura (Caelatura) companyoi* and the snail *Cleopatra bulimoides* as a function of time.

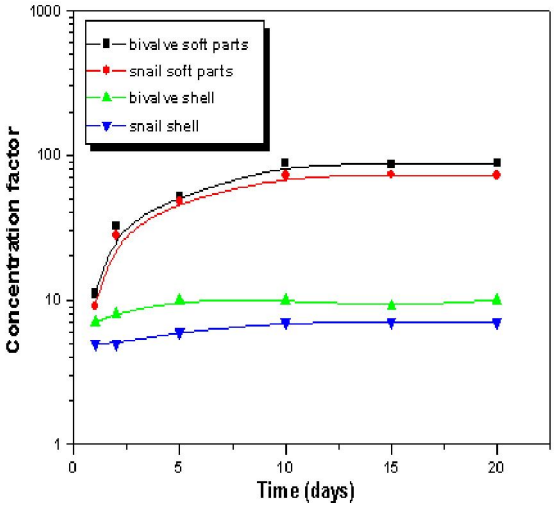

Fig. 2: Mean concenteration factor of Cadmium in soft parts and shell of the bivalve *Caelatura (Caelatura) companyoi* and the snail *Cleopatra bulimoides* as a function of time.

Fate of Cd and Cr

The toxic metals were accumulated mainly in the soft parts (more than 90%) while a small amount was adsorbed in the shells which was immediately released when the shells were immersed in 0.1M HCl for 5 minutes.

Toxicity of Cd and Cr (Figures 3-6)

Specimens of both bivalves and snails were subjected to different concentrations, from 1 to 20 mg/l in the case of cadmium and from 5 to 40 mg/l in the case of chromium. The estimated LC_{50} of Cd was 8.0 and 7.5 mg/l in the bivalve and snail, respectively. The corresponding values were 32.5 and 30.0 mg/l in the case of chromium. It could be concluded that cadmium is more toxic than chromium and that both investigated molluscs can tolerate higher concentrations of chromium than those of Cd.

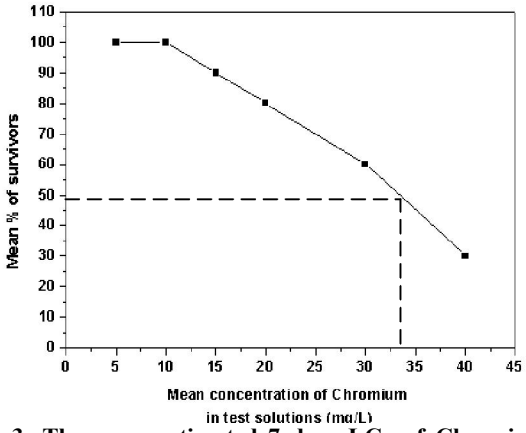

Fig.3: The mean estimated 7 days LC_{50} of Chromium for the mean percentage survivors of the bivalve *Caelatura (Caelatura) companyoi.*

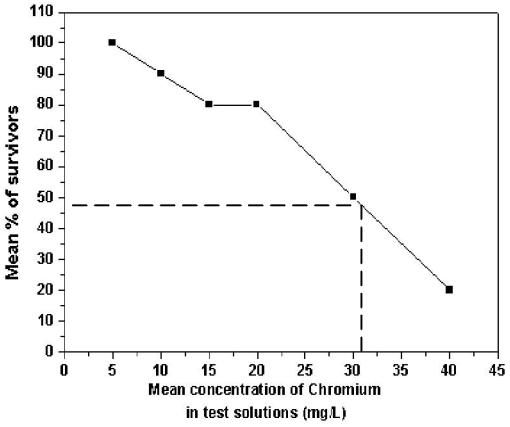

Fig.4: The mean estimated 7 days LC_{50} of Chromium for the mean percentage survivors of the snail *Cleopatra bulimoides*.

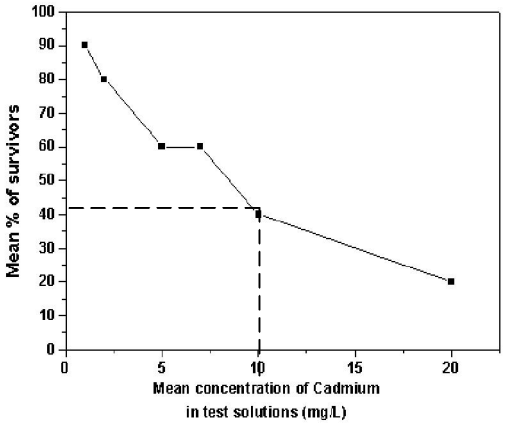

Fig.5: The mean estimated 7 days LC_{50} of Cadmium for the mean percentage survivors of the bivalve *Caelatura (Caelatura) companyoi.*

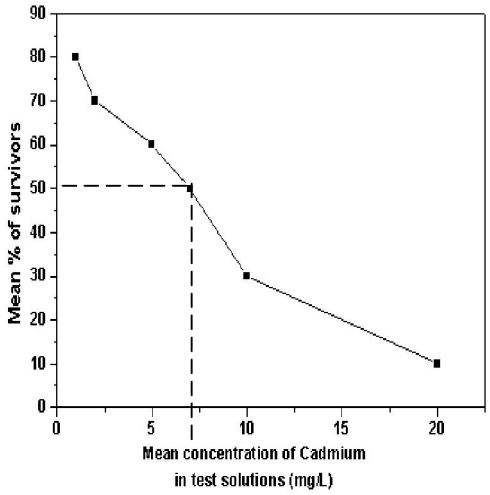

Fig. 6: The mean estimated 7 days LC_{50} of Cadmium for the mean percentage survivors of the snail *Cleopatra bulimoides.*

4. Discussion

In the present study, the freshwater molluscs *Caelatura (Caelatura) companyoi* and *Cleopatra bulimoides* were exposed to different concentrations of chromium and cadmium for twenty days in the laboratory to examine the effects of these heavy metals on their survival and their fate through their soft parts and shells. From the obtained results it is clear that the analysis of water and the investigated molluscs (soft parts and shells) from the field indicated that these organisms can accumulate Cd and Cr in high concentrations in their bodies, so they can be used as bioindicators for heavy metals

pollution in aquatic ecosystems. This is in agreement with the studies of Lopez-Artiguez *et al.* (1989), Brown and Louma (1995) and Burgos and Rainbow (2001) on some bivalves. Results showed that cadmium is much more toxic than chromium. This agrees with the finding of Shivaraj and Patil (1985) on the freshwater fish *Lepidocephalichthys guntea* and of Cain and Louma (1986) and Ibrahim *et al.* (1997) on some bivalves.

The present work indicates that accumulations of Cd and Cr in the soft parts of the examined molluscs increase as their concentrations in their medium increase at various immersion time intervals. This is in agreement with the findings of EL-Deek *et al.* (1994) on fishes, Hook and Fisher (2002) on copepods and Buschiazzoa *et al.* (2004) on oysters. Moreover, Hemelraad *et al.* (1986, 1990) reported that mortality of *Anodonta cygnea* due to Cd was low and not differing from a control group, for up to fifteen weeks, but strongly increased after the period.

Cadmium and chromium are very toxic (FAO, 1983; UNEP, 1984). The investigated bivalves and snails can tolerate and accumulate them to a great extent. Similar observations were given for other molluscs (Hemelraad and Zandee (1986); Hemelraad *et al*. (1990) and Brown and Louma, 1995).

Many workers found that the accumulation patterns of heavy metals are dependent on both uptake and elimination rates (Hakanson, 1984, Gomaa *et al.,* 1995). The pattern of both heavy metals accumulation in the present test animals was nearly similar. Some workers demonstrated that bivalves accumulate more cadmium than crustaceans and much more than fish (Howard and Nickless, 1977). Moreover, bivalves and other molluscs were found to be critical groups in the biological transport (Engel and Fowler, 1979, Frazier, 1979). Bivalve molluscs were also reported to withstand remarkably high metal concentrations in their environment. Cadmium, Zinc, Nickel, Copper, Lead and Mercury are accumulated to high tissue levels in both freshwater and marine mussels (Zadory, 1984; Hemelraad *et al.,* 1986 and 1987).

Conclusion

It could be concluded that the bivalve *Caelatura (Caelatura) companyoi* and the snail *Cleopatra bulimoides* could be considered as good bioindicators for pollution of heavy metals in aquatic ecosystems.

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Comparative Study on the Intestine of *Schilbe Mystus* and *Labeo Niloticus* in Correlation with their Feeding Habits

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Abstract: The present investigation aims to illustrate the morphological, histological and ultrastructural differences in the intestine of a carnivorous fish, *Schilbe mystus* and a herbivorous fish, *Labeo niloticus*, in correlation with their feeding habits. The histological examination of the mucosa of the intestine of both studied species revealed that it is built up of a simple columnar epithelium that contains goblet cells. Though these, are generally more numerous in the intestine of *Schilbe mystus* than in that of *Labeo niloticus*. The ultrastructural investigation of the mucosa of the duodenum and ileum of *Schilbe mystus* revealed that the enterocytes are tall columnar cells and possess well-developed microvilli, numerous mitochondria, microtubules and numerous lipid droplets. The ultrastructural examination of the mucosa of the ileum of *Labeo niloticus* revealed that the enterocytes are tall columnar and possess well-developed microvilli, numerous mitochondria, microtubules, a few endocytotic channels and aggregated chylomicrons. The enterocytes of the rectum of both studied species are tall columnar and are characterized by the presence of short and well-spaced microvilli, a well-developed vesiculotubular system, numerous microtubules and mitochondria. In addition, the rectal enterocytes of *Schilbe mystus* contain endocytotic vesicles. The intestinal muscularis of both studied species is well-developed and built up of unstriated muscle fibres, which are arranged into two layers: an inner circular layer and an outer longitudinal one. The rectal muscularis of both studied species is thicker than that of the rest of the intestine.
[Fawzy I. Amer; Suzan A. A. Naguib and Fawzia A. Abd El Ghafar Comparative Study on the, Intestine of *Schilbe Mystus* and *Labeo Niloticus* in Correlation with their Feeding Habits. Journal of American Science 2011;7(8):465-484].(ISSN: 1545-1003). http://www.americanscience.org.

Key words: *Schilbe mystus*, *Labeo niloticus,* feeding habits, intestine light and ultrastructure.

1. Introduction:

Al-Hussaini (1947) stated that the relative gut length of some marine teleosts is correlated to the diet in a consistent manner. He found that the herbivores possess the longest relative gut length, followed by omnivores, carnivores and finally planktivores with the shortest intestine. Elliott and Bellwood (2003) also postulated the same concept in three families of coral reef fishes. Nevertheless, they stated that the corallivores and omnivores typically possess the longest intestine, then herbivores and finally carnivores with the shortest intestine.

Yamamoto (1966), in *Salmo irideus* and *Carassius auratus*; Kayanja *et al.* (1975), in *Tilapia grahami*; Stroband (1977), in the juvenile of *Ctenopharyngodon idella*; Noaillac-Depeyre and Gas (1979), in *Perca fluviatilis*, and Anderson (1986), in *Girella tricuspidata* found that the height of the microvilli of the teleost enterocytes is gradually decreased from the anterior to the posterior parts of the intestine.

Kapoor *et al.* (1975) and Grau *et al.* (1992) stated that the main features of the carnivorous fish digestive tract are the presence of large stomach and short intestine.

Jilek (1979) stated that the intestinal mucosa of the filter feeder teleost, *Dorosoma cepedianum*, consists of two basic cell types, the goblet and columnar cells. He also stated that the intestine has a thin muscularis mucosa and the muscularis consists of two layers of the smooth type, an outer longitudinal and an inner circular muscle layers. Ünal *et al.* (2001) also postulated the same concept in *Chalcalburnus tarichi*.

Watanabe (1981), in some teleosts, and Anderson (1986) in *Girella tricuspidata*, elucidated that the intestine can be divided into an anterior lipid-absorbing region and a posterior region for absorbing macromolecular proteins and soluble nutrients.

MacDonald (1987), in the juvenile and adult Drover sole, *Solea solea*, and Caceci and Hrubec (1989 &1990), in the black mollie, *Poecilia* spp., stated that the apical microvilli characterize the epithelial cells of all regions of the gastro-intestinal tract.

Deplano *et al.* (1989) reported that the enterocytes of the proximal intestine of *Dicentrarchus labrax* contain two categories of lipids: the lipid granules or particles (chylomicrons) and free lipid droplets.

Grau *et al.* (1992) stated that the pyloric caeca of *Seriola dumerili*, which are located between the stomach and intestine, might be related to its feeding habit. In addition, they stated that the

muscularis is formed of three muscular layers throughout the whole length of the intestine, whereas only two layers are found in most teleost species. The same authors explained that the increase of the smooth muscular sheet could be related to the increase of intestinal motility, which would optimize the intestinal absorption in the carnivorous fish with irregular intake of large quantities of food. They also noticed that the rectum displays a deeply infolded mucosa and its muscularis is twice as thick as the intestinal muscular layer, which would contribute to the expulsion of the undigested materials through the anus.

Kuperman and Kuz'mina (1994) stated that there is probably a link between the feeding habits of the teleost fish in general and the structure of the enterocyte brush border.

Tibbetts (1997) observed that the digestive system of the snub-nosed garfish, *Arrhamphus sclerolepis krefftii*, lacks many of the alimentary modifications common in the alimentary tract of the herbivorous fishes such as, the acidic stomach, long intestine and pyloric caeca.

Albrecht *et al.* (2001) in *Leporinus friderici* and *Leporinus taeniofasciatus*, and Cinar and Senol (2006), in *Pseudophoxinus antalyae,* reported that the digestive tract has a four-layered general structure namely: mucosa, submucosa, muscularis and serosa, while the muscularis mucosa is absent.

Petrinec *et al.* (2005) stated that the mucosubstances in the mucosa of the digestive tract of *Esox lucius* and *Silurus glanis* are involved in lubrication and protection processes of the mucosa against acidity and enzymatic actions.

Materials and Methods

The freshwater Nile silver schilbeid catfish, *Schilbe mystus* (Linnaeus, 1758), (Order: Siluriformes, Family: Schilbeidae) and the freshwater Nile carp, *Labeo niloticus* (Forsskål, 1775), (Order: Cypriniformes, Family: Cyprinidae) were used in the present investigation.

1- Collection of the Samples:

The adult specimens of the silver schilbeid catfish, *Schilbe mystus*, ranging from 15-28 cm in length, and of the Nile carp, *Labeo niloticus*, ranging from 20-40 cm in length, were caught alive from the River Nile at different localities between Beni Suef and Giza. They were killed and dissected immediately in the field.

2-Morphological, Histological and Ultrastructural Studies:

Fresh adult specimens were carefully dissected to expose the digestive system. The gut length (LG) was measured as the distance from the oesophagus to the end of the rectum. The gut length was correlated with the standard length (LS) of the fish to obtain the relative gut length (RGL) of each species. Which is expressed by the following equation:

$$RGL = (LG).(LS)^{-1}$$

Then, small pieces of each intestinal region of both studied species were fixed in aqueous or alcoholic Bouin's fluid for 24 hrs. After fixation and washing, dehydration was carried out in ascending grades of ethyl alcohol. The materials were then cleared in terpineol and were embedded in molten paraplast. Sections of 5-7 µm thick were cut and were stained with Harris' haematoxylin and counterstained with eosin. Some sections were stained with Periodic Acid-Schiff reagent (PAS) and counterstained with haematoxylin to demonstrate the mucus-secreting cells. The sections were examined by a light microscope and measurements were carried out using the eyepiece micrometer, calibrated by the stage micrometer. Photomicrographs were made as required.

Small pieces of the different regions of the intestine, (the duodenum, ileum and rectum of *Schilbe mystus* and the ileum and rectum of *Labeo niloticus*) were immediately fixed in cold 3% glutaraldehyde solution in phosphate buffer (pH 7.2 to 7.4) or in a mixture of glutaraldehyde and formaldehyde for three hours to overnight at 4°C, then immersed in the phosphate buffer for 24 hrs. The materials were then washed thoroughly with phosphate buffer at least for three times, 10 minutes each, with shaking. The materials were then post-fixed in 2 % osmium tetroxide (OsO_4) for 1 hr in a dark place at 4°C. Then the materials were washed with phosphate buffer three times, 10 minutes each. The materials were dehydrated in ascending series of ethyl alcohol (up to the absolute), 10 minutes each, at 4°C. They were then cleared in pure propylene oxide for 15 minutes and transferred to a mixture of propylene oxide and epoxy resin.

Afterwards, the materials were infiltrated with pure epoxy resin for 4 hrs and embedded in fresh epoxy resin at 60°C in an oven under vacuum.

Resin blocks were cut with the ultramicrotome into semithin sections (1µm thick) and stained with toluidine blue. The slides were examined with the light microscope to select the suitable areas for ultrathin sectioning. Silver to pale golden ultrathin sections (600Å in thickness) were mounted on copper grids. The sections were stained in darkness with 5% uranyle acetate and lead citrate according to Reynolds (1963). Finally, the grids were then examined with JOEL 1200 EX II electron microscope at the Electron Microscope Unit in the Central Laboratory, Faculty of Science, Ain Shams

University and JOEL 100S electron microscope at the Electron Microscope Unit in the National Cancer Institute.

3. Results

1- Morphological Studies

A- *Schilbe mystus*:

The intestine of *Schilbe mystus* is relatively short with few small coils or loops. Posterior to the pyloric sphincter, the intestine can be distinguished into three regions: the duodenum, ileum and rectum. There are no obvious constrictions between these three different regions of the intestine.

The duodenum is the most anterior portion of the intestine and is a wide, short and straight tube**.** The ileum consists of a coiled tube, whose coils are attached with each other by the mesenteries. The rectum consists of a straight tube, which opens posteriorly by the anus.

The gut length is short and the relative gut length varies from 1.4 to 2.3 cm (Table 1).

B- *Labeo niloticus*:

The intestine of *Labeo niloticus* occupies the greater part of the abdominal cavity and is formed of an extremely long and thin-walled tube. The intestine is distinguished into two regions: a proximal ileum and a distal rectum.

The ileum consists of a long, coiled thin-walled tube. The coils of the ileum are numerous, long and held together by the mesenteries, which possess scattered red patches of the spleen. These coils are arranged in the horizontal plane within the abdominal cavity. On the other hand, the rectum consists of a straight uncoiled tube, which opens by the anus. There is no visible marked line separating the two different regions of the intestine proper.

The gut is remarkably long and the relative gut length varies from 16.6 to 21.9 cm (Table 1).

Table (1): The standard length (LS), the gut length (LG) and the relative gut length (RGL), which equals (LG). $(LS)^{-1}$, of *Schilbe mystus* and *Labeo niloticus*. (The total number of the specimens examined for each species = 10).

Schilbe mystus			*Labeo niloticus*		
LS (cm)	**LG (cm)**	**RGL**	**LS (cm)**	**LG (cm)**	**RGL**
13	20	1.5	27.5	455	16.6
18	25.5	1.4	29	560	19.3
18	27.5	1.5	30	545	18.2
18	30	1.7	30	570	19.0
19	43	2.3	31	596	19.2
19.5	31	1.6	32	621	19.4
21	32.5	1.6	32	590	18.4
21.5	50	2.3	32	615	19.2
22	30.5	1.4	32.5	610	18.8
24	46	1.9	35	765	21.9
The RGL mean value = 1.7			The RGL mean value = 19		

2- Histological Studies

The intestinal wall of both studied species is constructed of the main four layers: the mucosa, submucosa, muscularis and serosa**.**

The lymphocytes are numerous and are scattered in the mucosal epithelium and the lamina propria of the whole intestine**.** They are easily distinguished by their scanty amount of cytoplasm and rounded centrally located nuclei**.**

A-The intestine of *Schilbe mystus*

1- The duodenum:

The duodenal wall will be described in details, then the description of the rest of the intestinal wall is focused on the structural differences.

i- The mucosa:

The mucosa of the duodenum is thrown up into long highly branched villi (Fig.1). These villi are covered with a simple columnar epithelium that contains numerous goblet cells (Fig. 2).

The columnar cells of the duodenal mucosa have the typical structure of the absorptive cells. They are tall columnar cells with a prominent brush border (Figs. 2 and 3). The columnar cells possess a finely granulated eosinophilic cytoplasm and oval nuclei, whose long axes are perpendicular to the extremely thin basement membrane. The nuclei are situated in the basal half of the cells at nearly the same levels (Fig. 3). The cytoplasm, especially the apical part, possesses numerous aggregations of lipid droplets (Fig. 4).

The goblet cells:

The goblet cells are pyriform in shape with two distinct parts: the apical swollen and the basal elongated parts**.** The apical swollen part contains secretory granules, while the basal elongated part contains an oval nucleus**.** The swollen part possesses a single pore on the luminal surface of the duodenal epithelium (Figs. 2 and 4).

The lamina propria:

The lamina propria consists of a loose connective tissue that lies beneath the mucosal epithelium and supports its villi (Figs. 2 and 3).

ii- The submucosa

The submucosa is an extremely thin layer of loose connective tissue, which is in continuation with that of the lamina propria (Figs. 2 and 3).

iii- The muscularis

The muscularis is thin and consists of unstriated muscle fibres arranged in two layers: an outer longitudinal and an inner circular muscle layers (Fig. 5).

iv- The serosa

The serosa consists of a simple squamous epithelium with flattened centrally located nuclei (Fig. 5).

2- Ileum:

i- The mucosa

The mucosa of the ileum is thrown up into numerous long simple villi (Fig. 6). The mucosa is lined by a simple columnar epithelium, which contains numerous goblet cells (Fig. 7). The columnar cells have the typical structure of the absorptive cells; they are tall columnar and have a prominent brush border. These columnar cells possess a highly vacuolated eosinophilic cytoplasm and oval basally located nuclei whose long axes are perpendicular to the extremely thin basement membrane. The cytoplasmic vacuoles are lipid aggregations, which are mainly concentrated in the apical cytoplasm (Fig. 7).

iii- The muscularis

The muscularis of the ileum is thin anteriorly, but is gradually increased in thickness posteriorly. The muscularis consists of unstriated muscle fibres arranged in two layers: an outer longitudinal layer and an inner circular one (Fig. 8).

3- The rectum:

i- The mucosa

The mucosa of the rectum is organized into numerous long simple villi (Fig. 9). The mucosa is built up of a simple columnar epithelium which contains numerous goblet cells. The columnar cells of the rectum are tall and have a prominent darkly stained brush border. These cells have a homogeneous finely granulated eosinophilic cytoplasm and oval nuclei, located in the basal half of the cells nearly at the same level. The long axes of the nuclei are perpendicular to the basement membrane (Fig. 10).

iii- The muscularis

The muscularis of the rectum is thick and well developed. The muscularis consists of unstriated muscle fibres arranged in two layers: an outer longitudinal and an inner circular muscle layers. The inner circular layer is more developed than the outer longitudinal one (Fig. 9).

B - The intestine of *Labeo niloticus*:

1- The ileum:

i- The mucosa

The mucosa of the ileum is thrown up into simple villi, which are long with tapering ends anteriorly and become shorter and broader posteriorly (Figs. 11 and 12). The mucosa consists of a simple columnar epithelium, which contains numerous goblet cells. The columnar cells have the typical structure of the absorptive cells; they are tall columnar and have a prominent brush border, which is less developed than that of the intestinal swelling. These columnar cells possess a homogeneous eosinophilic cytoplasm and oval nuclei, whose long axes are perpendicular to the basement membrane (Figs. 11and 13).

iii- The muscularis

The muscularis of the ileum consists of unstriated muscle fibres arranged in two layers: an outer longitudinal and an inner circular muscle layers (Figs. 11and 12).

2- The rectum:

i- The mucosa

The mucosa of the rectum is thrown up into simple and branched villi with broad ends (Fig. 14). The mucosa is lined by a simple columnar epithelium containing goblet cells. The columnar cells of the rectum are tall and have a prominent brush border, which is less developed than that of the intestinal swelling and ileum. The columnar cells have a homogeneous finely granulated eosinophilic cytoplasm and oval nuclei, whose long axes are perpendicular to the basement membrane (Fig. 15).

iii- The muscularis

The muscularis of the rectum is well developed and consists of unstriated muscle fibres arranged in two layers: an outer longitudinal and an inner circular muscle layers (Fig. 16).

3- Ultrastructural Studies

a- The enterocytes of the duodenum

The enterocytes of the duodenum are tall columnar cells (about 33 µm in average height), which rest on the basement membrane. The apical cell membrane bears numerous regularly arranged cytoplasmic projections, the microvilli, which form the brush border. These microvilli are covered by the glycocalyx and the upper part of some of them is dense and forms spherical bleb, the capitulum. The mean length of the microvilli is about 2.4 µm and mean diameter is about 0.12 µm. The apical cytoplasm, just below the microvilli, has no cellular organelles and forms the terminal web (Fig. 17).

The cells are heavily loaded with lipid droplets of variable sizes (the diameter varies from 0.7 to 3.8 µm). These droplets are rounded in shape and are scattered throughout the cell cytoplasm and sometimes coalesce with each other (Fig. 17).

The Golgi apparatus is formed of numerous parallel stacks and lies near the nucleus (Fig. 18).

The rough endoplasmic reticulum consists of numerous long parallel tubules, which are studded with numerous ribosomes (Fig. 19). On the other hand, the smooth endoplasmic reticulum is poorly developed. Numerous free ribosomes are scattered throughout the cell cytoplasm (Fig. 18).

The mitochondria are numerous, electron-dense, polymorphic (rounded, oval and elongated in shape) and are of variable sizes (the mean diameter is about 0.3 µm and the mean long axis is about 1.3 µm). The inner mitochondrial membrane forms numerous lamellar cristae (Fig. 19). The mitochondria are accumulated mainly in two regions: the apical cytoplasm beneath the terminal web and the infranuclear region.

The enterocyte is characterized by the presence of numerous microtubules, which are scattered in the cell cytoplasm and run parallel to the long axis of the cell (Fig. 20). The mean diameter of these microtubules is about 0.02 µm (22 nm).

The nuclei of the enterocytes are oval in shape (the long axis is about 6µm) and each one contains a prominent centrally located nucleolus. The nuclear envelope is a double membrane consisting of the outer and inner nuclear membranes that encompass the nucleoplasm. The nuclear pores are found at intervals in the nuclear envelope. The euchromatin is represented by the pale granular areas of the nucleoplasm. On the other hand, the heterochromatin is represented by small and dense granular patches scattered throughout the nucleoplasm and attached to the inner side of the inner nuclear membrane (Fig. 21).

The lateral cell membranes form smooth contact with the adjacent cells and lack interdigitations. The apical ends of these lateral walls of the adjacent cells join together by the junctional complexes (Fig. 17).

b- The enterocytes of the ileum

The enterocytes of the ileum are tall columnar cells (about 35 µm in average height), which rest on the basement membrane. The apical cell membrane carries numerous, juxtaposed and regularly arranged cytoplasmic projections, the microvilli, which form the brush border (Fig. 22). These microvilli are covered by the glycocalyx and the upper part of some of them is dense and forms spherical bleb, the capitulum. The mean length of the microvilli is about 2 µm and its mean diameter is about 0.09 µm. The apical cytoplasm, just below the microvilli, has no cellular organelles and forms the terminal web (Fig. 23).

The cell cytoplasm contains numerous lipid droplets, which are mainly rounded in shape with variable sizes (the diameter varies from 0.5 to 2.7 µm). These lipid droplets are scattered throughout the cell cytoplasm, especially in the supranuclear region (Fig. 22).

The Golgi apparatus is found in a supranuclear position and is formed of numerous parallel stacks (Fig. 24).

The endoplasmic reticulum is well developed and consists of two types: the rough and smooth endoplasmic reticula. The rough endoplasmic reticulum consists of tubules and cisternae, which are studded with numerous ribosomes (Fig. 23). On the other hand, the smooth endoplasmic reticulum consists of smooth cisternae. The free ribosomes are abundant and scattered throughout the cell cytoplasm (Fig. 24).

The mitochondria are numerous, electron-dense, polymorphic (rounded, oval, elongated and ring-like in shape) and with variable sizes (the mean diameter is about 0.3 µm and the mean long axis is about 1.3 µm). The inner mitochondrial membrane forms numerous lamellar cristae (Fig. 25). The mitochondria are accumulated mainly in two regions: the apical cytoplasm beneath the terminal web and the infranuclear region.

The enterocytes are characterized by the presence of numerous microtubules, which are scattered in the cell cytoplasm and run parallel to the long axis of the cell. The mean diameter of these microtubules is about 0.02 µm (23 nm). These

microtubules are seen connected to the lateral cell membranes (Fig. 26)

The nucleus is oval in shape (the long axis is about 6 µm) and possesses a prominent nucleolus (Fig. 22). The nuclear envelope is a double membrane consisting of the outer and inner nuclear membranes that encompass the nucleoplasm. The nuclear pores are found at intervals in the nuclear envelope. The euchromatin is represented by the pale granular areas of the nucleoplasm, whereas the heterochromatin is represented by small dark granular patches scattered throughout the nucleoplasm and attached to the inner side of the inner nuclear membrane (Fig. 26).

The lateral cell membranes form smooth contacts with the adjacent cells and lack interdigitations. The apical ends of these lateral walls of the adjacent cells join together by the junctional complexes (Fig. 23).

c- The enterocytes of the rectum

The enterocytes of the rectum are tall columnar cells (about 35 µm in average height), which rest on the basement membrane. The apical cell membrane carries numerous short, well-spaced and regularly arranged cytoplasmic projections, the microvilli, which form the brush border (Fig. 27). These microvilli are covered by a prominent glycocalyx and the upper part of some of them is dense and forms spherical bleb, the capitulum. The mean length of the microvilli is about 0.5 µm and their mean diameter is about 0.1 µm. The terminal web contains a few number of the endocytotic vesicles (Fig. 28). The lipid droplets are totally absent from the cell cytoplasm.

The apical cell cytoplasm, just beneath the terminal web, contains the vesiculotubular system, which consists of numerous oval or rounded vesicles with electron-dense outlines (Fig. 28). These vesicles have variable sizes (the long axis varies from 0.1 to 0.4 µm). A few number of these vesicles are scattered throughout the cell cytoplasm.

The Golgi apparatus is found in a supranuclear position and is formed of one or more sets of numerous parallel stacks and small vesicles (Fig. 29).

The endoplasmic reticulum consists of two types: the rough and smooth endoplasmic reticula. The rough endoplasmic reticulum is well developed and consists of long tubules, which are studded with numerous ribosomes. On the other hand, the smooth endoplasmic reticulum consists of a few number of smooth cisternae. The free ribosomes are abundant and are scattered throughout the cell cytoplasm (Fig. 29).

The mitochondria are numerous, electron-dense, rounded or elongated in shape and are of variable sizes (the mean diameter is about 0.3 µm and the mean long axis is about 1.1 µm). The inner mitochondrial membrane forms numerous lamellar cristae (Fig. 30). The mitochondria are accumulated mainly in two regions: the supranuclear, just beneath the vesicles zone, and infranuclear regions.

The cytoplasm of the enterocyte is characterized by the presence of numerous lysosomes, which are surrounded by distinct membranes (Fig. 30).

The enterocytes are characterized by the presence of numerous microtubules and microfilaments, which are scattered in the cell cytoplasm. The microtubules run parallel to the long axis of the cell, with the mean diameter about 0.02 µm (22 nm) (Fig. 30). The microfilaments are scattered throughout the cell cytoplasm and even in the region of the terminal web (Fig. 28).

The nuclei of the enterocytes are large, oval in shape (the long axis is about 8 µm) and each one contains a prominent centrally located nucleolus (Fig. 27). The nuclear envelope is a double membrane consisting of the outer and inner nuclear membranes that encompass the nucleoplasm. The chromatin consists mainly of the euchromatin, which is represented by the pale granular areas of the nucleoplasm, and the heterochromatin which is poorly developed and is represented by the small dark granular areas (Fig. 29).

The lateral cell membranes form smooth contacts with the adjacent cells and lack interdigitations. The apical ends of these lateral walls of the adjacent cells join together by the junctional complexes (Fig. 27).

a- The enterocytes of the ileum

The enterocytes are tall columnar cells (about 54 µm in average height), which rest on the basement membrane. The apical cell membrane bears numerous and regularly arranged cytoplasmic projections, the microvilli, which form the brush border (Fig. 31). These microvilli are covered by the glycocalyx and the upper part of some of them is dense and forms spherical bleb, the capitulum. The mean length of the microvilli is about 1.3 µm and their mean diameter is about 0.14 µm. The apical cytoplasm, just below the microvilli, has no cellular organelles and forms the terminal web. Invaginations of the apical cell membrane are frequently seen among the microvilli, forming the endocytotic channels (Fig. 32).

The enterocytes cytoplasm and intercellular spaces contain small lipid chylomicrons (Fig. 33).

The Golgi apparatus is located near the nucleus and is formed of one or two sets of parallel stacks and vesicles (Fig. 33).

The rough endoplasmic reticulum is well developed and consists of numerous, long and parallel tubules (Fig. 33). On the other hand, the smooth endoplasmic reticulum consists of a few short smooth tubules and cisternae (Fig. 32). The free ribosomes are abundant and are scattered throughout the cell cytoplasm (Fig. 33).

The mitochondria are numerous, electron-dense, polymorphic (rounded, oval, elongated and ring-like in shape) and are of variable sizes (the mean diameter is about 0.3 µm and the mean long axis is about 1.1 µm). The inner mitochondrial membrane forms numerous lamellar cristae. They are mainly aggregated at the basal part of the cell (Fig. 34).

The cytoplasm is characterized by the presence of numerous lysosomes, which are surrounded by distinct membranes (Fig. 31).

The enterocyte contains numerous microtubules, which are scattered in the cytoplasm and run parallel to the lateral cell membranes. The microtubules are usually seen joining the lateral cell membranes (Fig. 33). The mean diameter of these microtubules is about 0.025 µm (25 nm).

The nuclei are large, oval in shape (the long axis is about 8.5 µm) and each one contains a prominent centrally located nucleolus (Fig. 31). The nuclear envelope is a double membrane consisting of the outer and inner nuclear membranes that encompass the nucleoplasm. The chromatin is represented by the euchromatin, which is formed of the pale granular areas of the nucleoplasm and the heterochromatin which is represented by the dark granular areas of the nucleoplasm (Fig. 35).

The lateral cell membranes form smooth contacts with the adjacent cells and lack interdigitations. The apical ends of these lateral walls of the adjacent cells join together by the junctional complexes (Figs. 31 and 32). Moreover, the basal cell membrane is extremely infolded and is in contact with the conspicuous basement membrane (Fig. 34).

b- The enterocytes of the rectum

The enterocytes are tall columnar cells (about 43 µm in average height), which rest on the basement membrane. The apical cell membrane bears short, well-spaced and regularly arranged cytoplasmic projections, the microvilli, which form the brush border (Fig. 36). These microvilli are covered by the glycocalyx. The mean length of the microvilli is about 0.2 µm and their mean diameter is about 0.15 µm. The apical cytoplasm, just below the microvilli, has no cellular organelles and forms the terminal web (Fig. 37).

The Golgi apparatus is well developed; it is found in a supranuclear position and is formed of one or more sets of numerous parallel stacks (Fig. 37).

The vesiculotubular system is well developed and is made up of numerous smooth vesicles and tubules with dense outlines. They are scattered throughout the cell cytoplasm, especially at the apical region (Fig. 37). The mean diameter of the vesicles is about 0.2 µm, while the mean diameter of the tubules is about 0.06 µm.

The rough endoplasmic reticulum consists of numerous long and parallel tubules, which are scattered in the cytoplasm and surround the nucleus. On the other hand, the smooth endoplasmic reticulum consists of smooth and wide tubules and cisternae (Fig. 37). The free ribosomes are abundant and scattered throughout the cell cytoplasm (Figs. 37 and 38).

The mitochondria are electron-dense, rounded or elongated in shape and have variable sizes (the mean diameter is about 0.6 µm and the mean long axis is about 1.2 µm). The inner mitochondrial membrane forms numerous lamellar cristae (Fig. 39). They are scattered throughout the cell cytoplasm (Fig. 36) and are also aggregated in the basal regions of the enterocytes (Fig. 39).

The cytoplasm of the enterocyte is characterized by the presence of numerous lysosomes, which are surrounded by distinct membranes (Figs. 36 and 37).

The enterocyte contains numerous microtubules, which are scattered in the cytoplasm and run parallel to the lateral cell membrane (Fig. 38). The mean diameter of these microtubules is about 0.025 µm (25 nm).

The nucleus is oval in shape (the long axis is about 7 µm) and with a prominent nucleolus. The nuclear envelope is a double membrane consisting of the outer and inner nuclear membranes that encompass the nucleoplasm. The chromatin is represented mainly by the euchromatin, which is formed of the pale granular areas of the nucleoplasm. On the other hand, the heterochromatin is poorly developed and consists of small dark granular patches at the inner sides of the inner nuclear membrane (Fig. 40).

The lateral cell membranes form smooth contacts with the adjacent cells and lack interdigitations. The apical ends of these lateral cell membranes of the adjacent cells join together by the junctional complexes (Fig. 36).

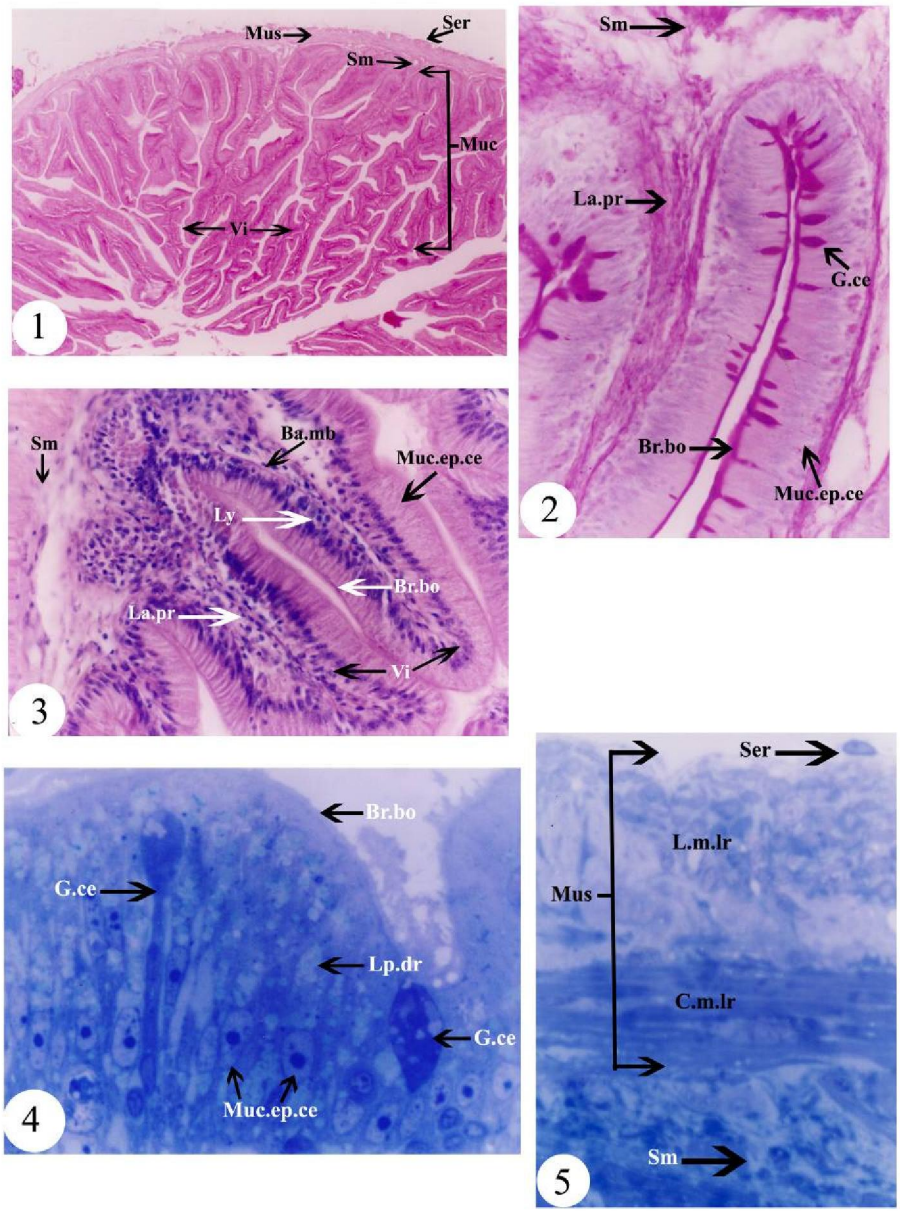

Fig. (1): Photomicrograph of T. S. of the duodenum of *Schilbe mystus* showing the mucosa, submucosa, muscularis, serosa and villi. H & E stain, X 45.

Fig. (2): Photomicrograph of T. S. of the duodenum of *Schilbe mystus* showing the mucosal epithelial cells, goblet cells, brush border, lamina propria and submucosa. PAS & H stain, X 485.

Fig. (3): Photomicrograph of T. S. of the duodenum of *Schilbe mystus* showing the villi, mucosal epithelial cells, lymphocytes, brush border, basement membrane, lamina propria and submucosa. H & E stain, X 450.

Fig. (4): Photomicrograph of a semithin section of the duodenum of *Schilbe mystus* showing the apical parts of the mucosal epithelial cells with lipid droplets and brush border and goblet cells. Toluidine blue stain, X 1340.

Fig. (5): Photomicrograph of a semithin section of the duodenum of *Schilbe mystus* showing the submucosa, muscularis, serosa circular muscle layer and longitudinal muscle layer. Toluidine blue stain, X 1210.

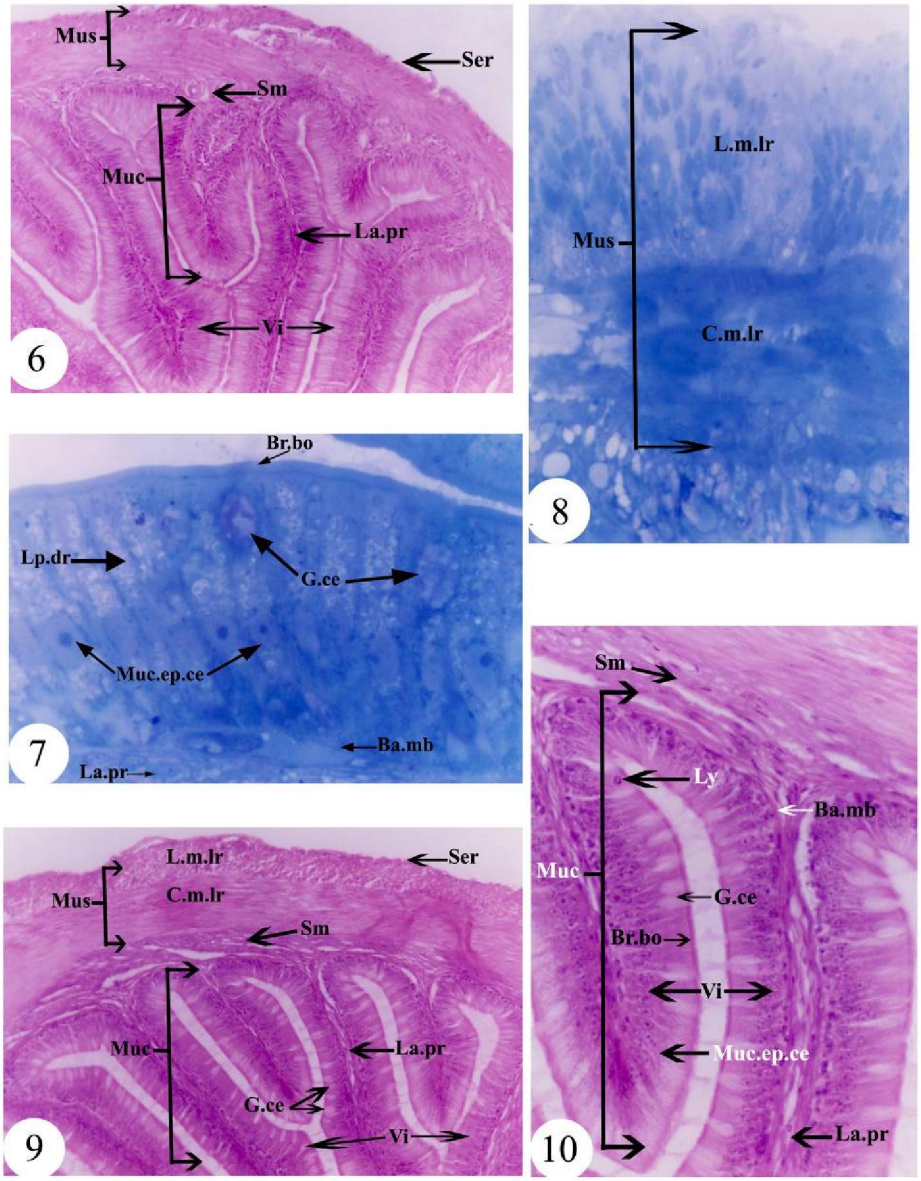

Fig. (6): Photomicrograph of T. S. of the ileum of *Schilbe mystus* showing the mucosa, submucosa, muscularis, serosa, villi and lamina propria. H & E stain, X 180.

Fig. (7): Photomicrograph of a semithin section of the ileum of *Schilbe mystus* showing the mucosal epithelial cells with lipid droplets and brush border, goblet cells, basement membrane and lamina propria. Toluidine blue stain, X 1290.

Fig. (8): Photomicrograph of a semithin section of the ileum of *Schilbe mystus* showing the muscularis with circular and longitudinal muscle layers. Toluidine blue stain, X 1350.

Fig. (9): Photomicrograph of T. S. of the rectum of *Schilbe mystus* showing the mucosa, submucosa, muscularis, serosa, villi, goblet cells, lamina propria, circular muscle layer and longitudinal muscle layer. H & E stain, X 190.

Fig. (10): Enlarged portion of Fig. (9) showing the mucosa, submucosa, villi, mucosal epithelial cells, goblet cells, lymphocytes, brush border, basement membrane and lamina propria. H & E stain, X 490.

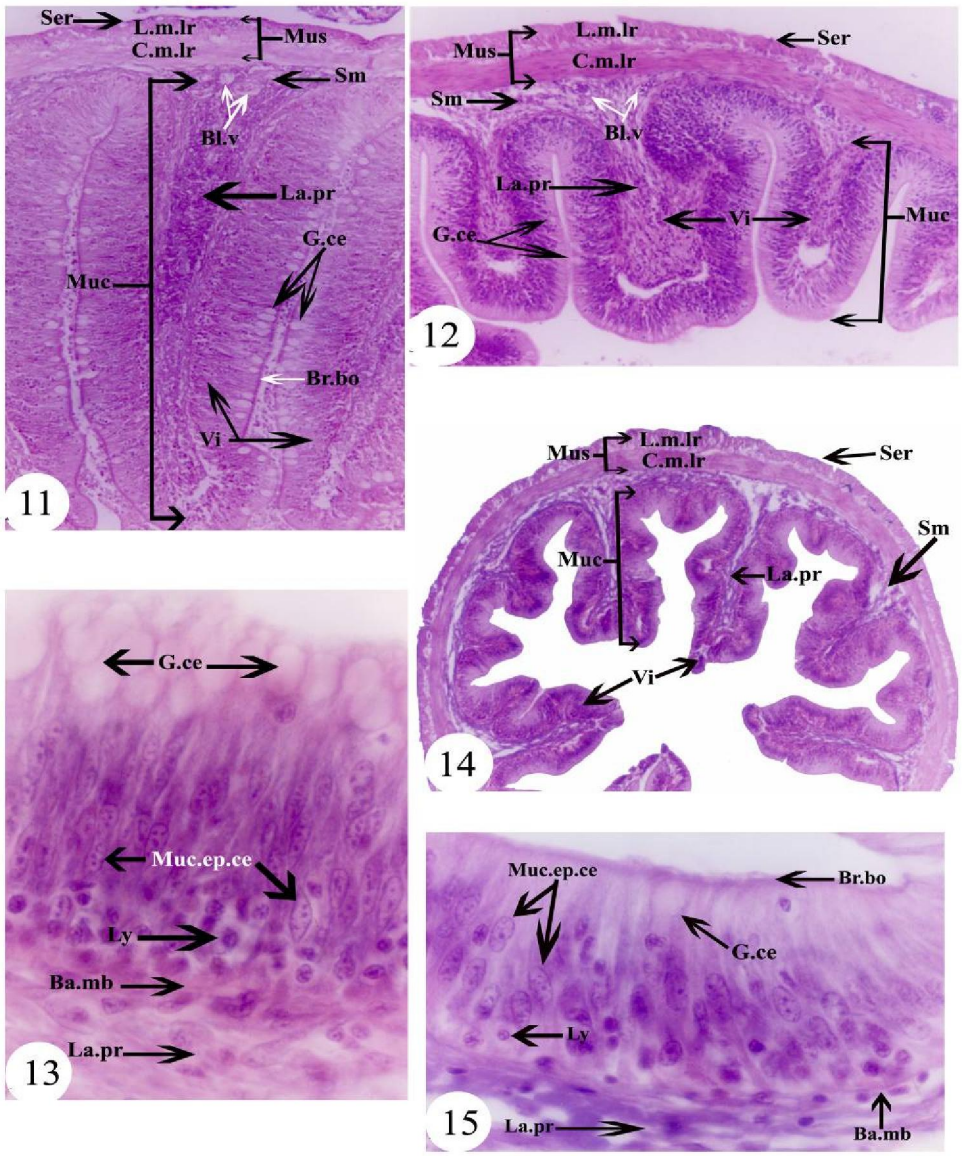

Fig. (11): Photomicrograph of T. S. of the anterior part of the ileum of *Labeo niloticus* showing the mucosa, submucosa, muscularis, serosa, villi, goblet cells, brush border, lamina propria, blood vessels, circular muscle layer and longitudinal muscle layer. H & E stain, X 200.

Fig. (12): Photomicrograph of T. S. of the posterior part of the ileum of *Labeo niloticus* showing the mucosa, submucosa, muscularis, serosa, villi, goblet cells, lamina propria, blood vessels, circular muscle layer and longitudinal muscle layer. H & E stain, X 190.

Fig. (13): Photomicrograph of L. S. of the ileum of *Labeo niloticus* showing the mucosal epithelial cells, goblet cells, lymphocytes, basement membrane and lamina propria. H & E stain, X 1360.

Fig. (14): Photomicrograph of T. S. of the rectum of *Labeo niloticus* showing the mucosa, submucosa, muscularis, serosa, villi, lamina propria, circular muscle layer and longitudinal muscle layer. H & E stain, X 110.

Fig. (15): Enlarged portion of Fig. (14) showing the mucosal epithelial cells, goblet cell, lymphocytes, brush border, basement membrane and lamina propria. H & E stain, X 1270.

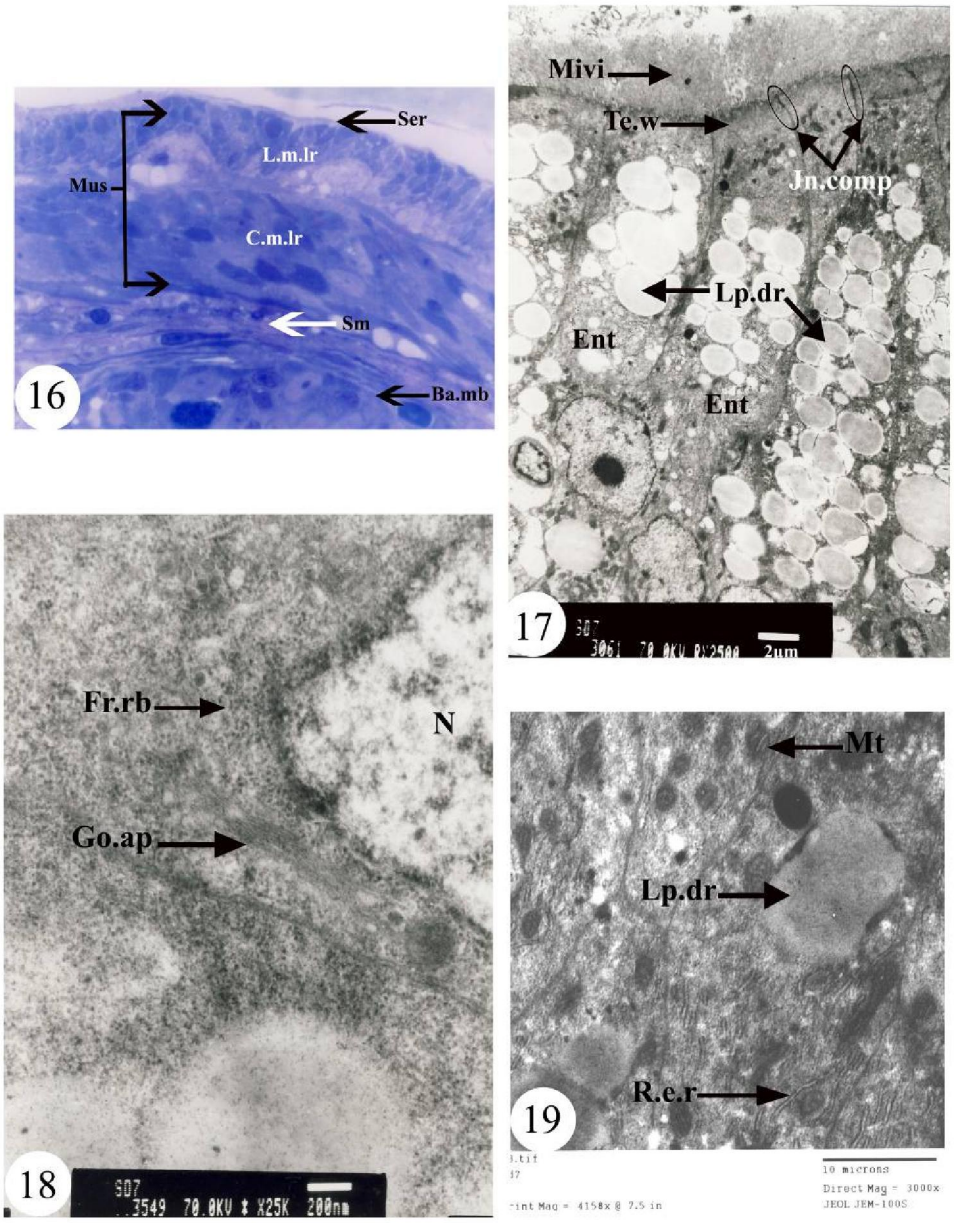

Fig. (16): Photomicrograph of a semithin section of the rectum of *Labeo niloticus* showing the submucosa, muscularis, serosa, basement membrane, circular muscle layer and longitudinal muscle layer. Toluidine blue stain, X 1150.

Fig. (17): Electron micrograph of the duodenal mucosa of *Schilbe mystus* showing the enterocytes, microvilli, terminal web, junctional complexes and lipid droplets.

Fig. (18): Electron micrograph of the duodenal enterocytes of *Schilbe mystus* showing the Golgi apparatus, free ribosomes and nucleus.

Fig. (19): Electron micrograph of the duodenal enterocytes of *Schilbe mystus* showing the lipid droplets, rough endoplasmic reticulum and mitochondria.

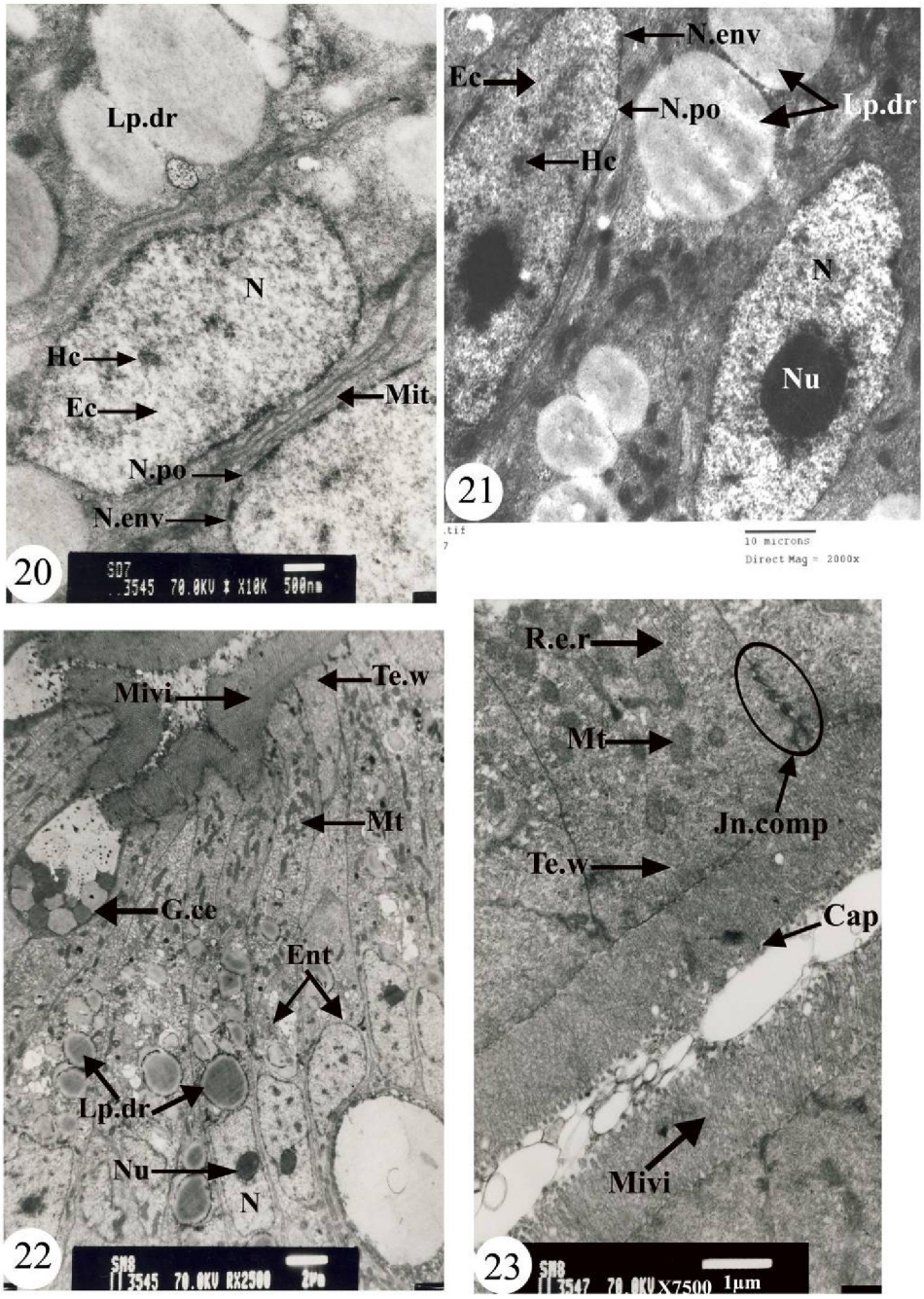

Fig. (20): Electron micrograph of the duodenal enterocytes of *Schilbe mystus* showing the microtubules, nucleus, euchromatin, heterochromatin, nuclear envelope and nuclear pore.

Fig. (21): Electron micrograph of the duodenal enterocytes of *Schilbe mystus* showing the lipid droplets, nucleus, nucleolus, euchromatin, heterochromatin, nuclear envelope and nuclear pore.

Fig. (22): Electron micrograph of the mucosa of the ileum of *Schilbe mystus* showing the enterocytes, goblet cell, microvilli, terminal web, mitochondria, lipid droplets, nucleus and nucleolus.

Fig. (23): Electron micrograph of the mucosa of the ileum of *Schilbe mystus* showing the apical parts of the enterocytes, microvilli, capitulum, terminal web, junctional complex, rough endoplasmic reticulum and mitochondria.

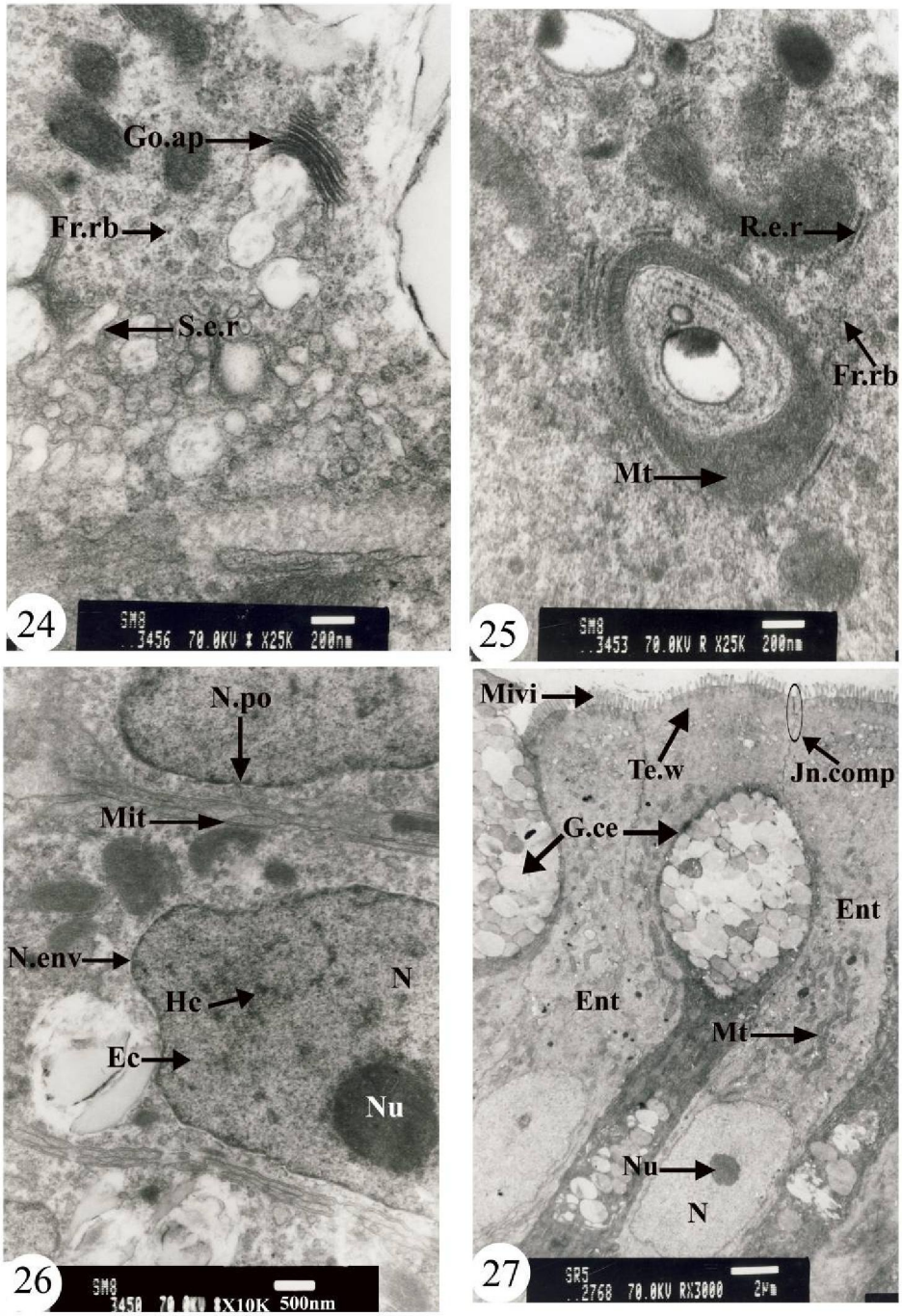

Fig. (24): Electron micrograph of the enterocytes of the ileum of Schilbe mystus showing the Golgi apparatus, free ribosomes and smooth endoplasmic reticulum.

Fig. (25): Electron micrograph of the enterocytes of the ileum of Schilbe mystus showing the rough endoplasmic reticulum, free ribosomes and mitochondria.

Fig. (26): Electron micrograph of the enterocytes of the ileum of Schilbe mystus showing the microtubules, nucleus, nucleolus, euchromatin, heterochromatin, nuclear envelope and nuclear pore.

Fig. (27): Electron micrograph of the rectal mucosa of Schilbe mystus showing the enterocytes, goblet cells, microvilli, terminal web, junctional complex, mitochondria, nucleus and nucleolus.

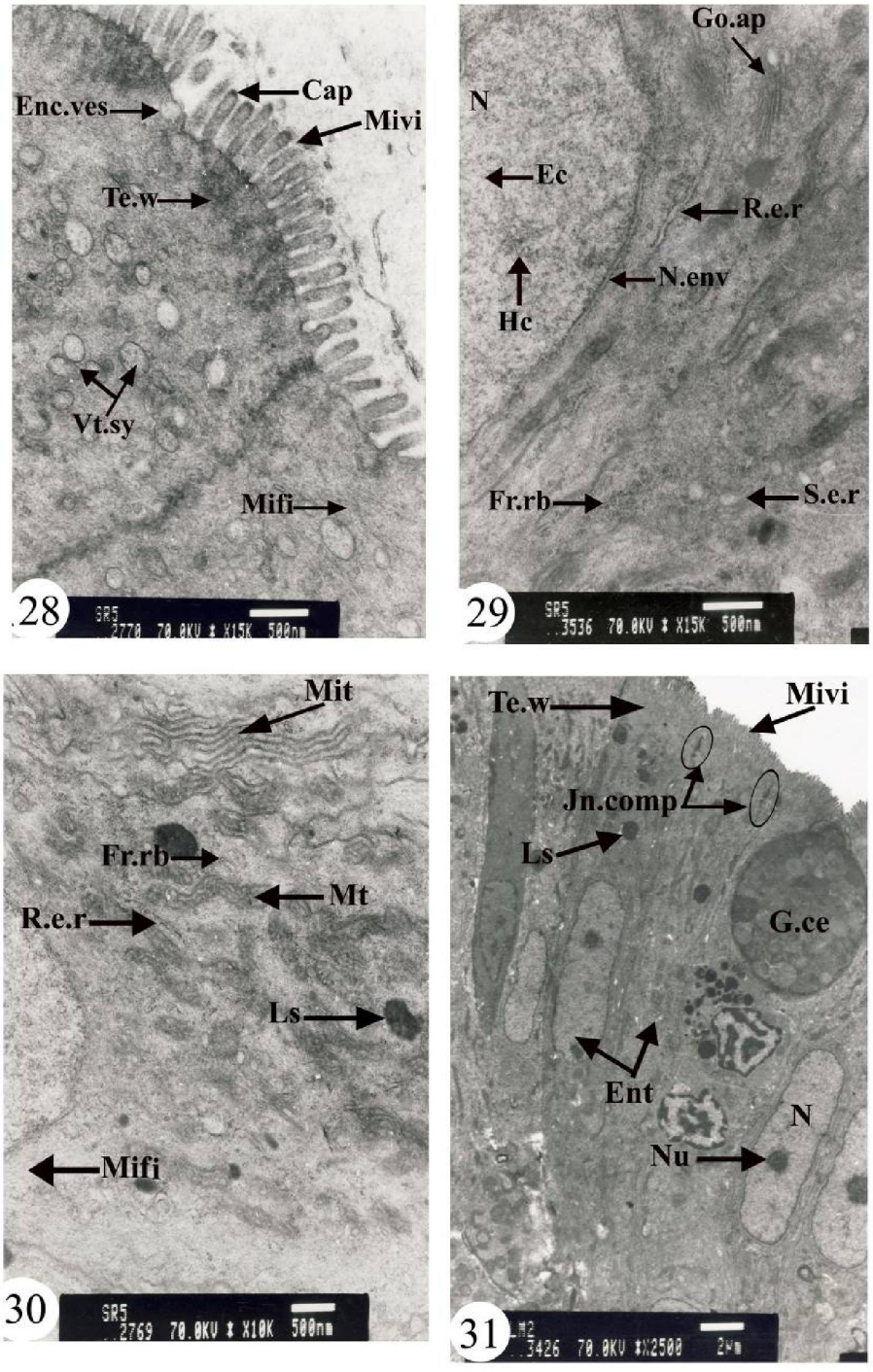

Fig. (28): Electron micrograph of the apical parts of the rectal enterocytes of Schilbe mystus showing the microvilli, capitulum, terminal web, endocytotic vesicles, vesiculotubular system and microfilaments.

Fig. (29): Electron micrograph of the rectal enterocyte of Schilbe mystus showing the Golgi apparatus, rough and smooth endoplasmic reticula, free ribosomes, nucleus, euchromatin, heterochromatin and nuclear envelope.

Fig. (30): Electron micrograph of the rectal enterocyte of Schilbe mystus showing the microtubules, microfilaments, rough endoplasmic reticulum, free ribosomes, lysosomes and mitochondria.

Fig. (31): Electron micrograph of the mucosa of the ileum of Labeo niloticus showing the enterocytes, goblet cell, microvilli, terminal web, junctional complexes, lysosomes, nucleus and nucleolus.

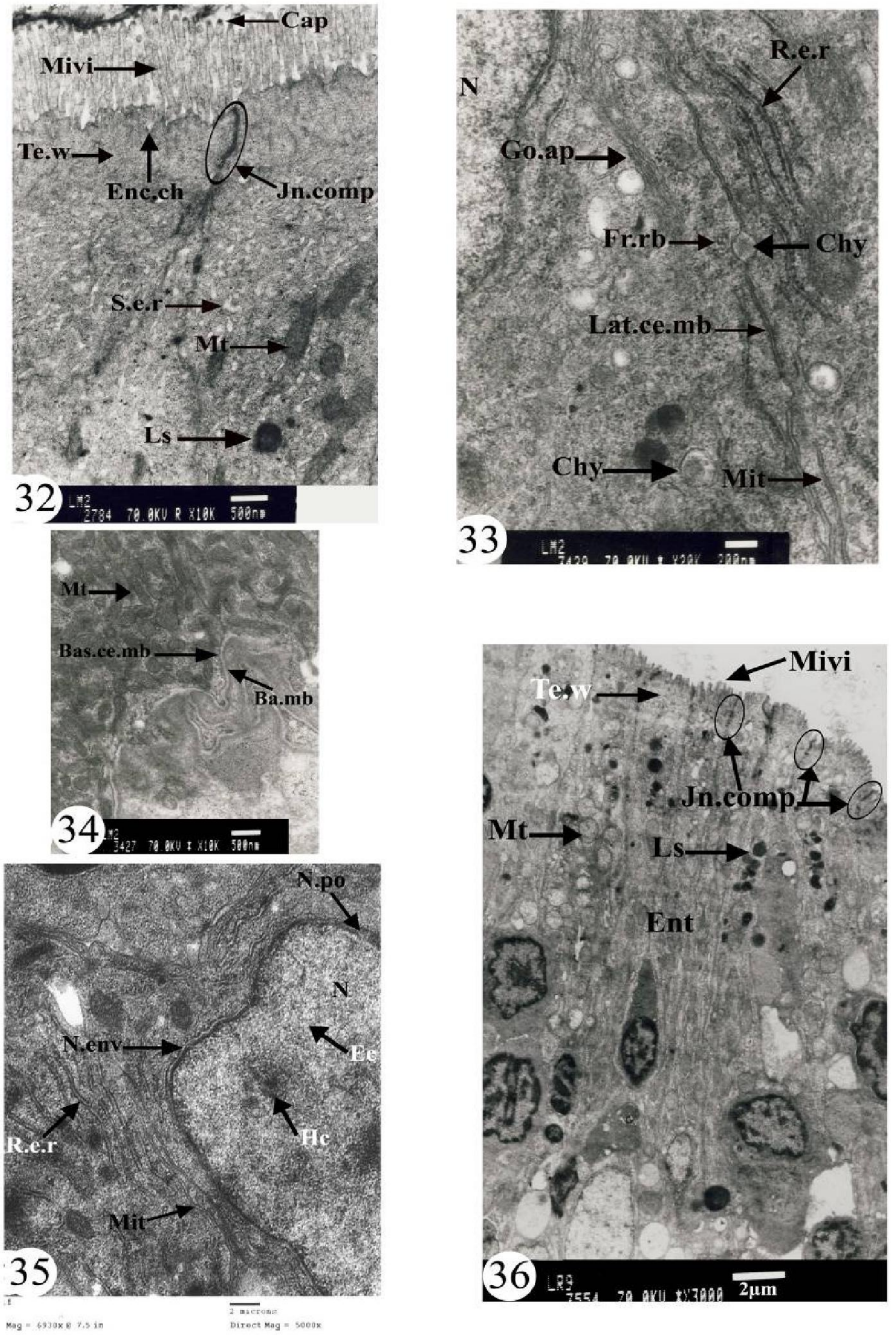

Fig. (32): Electron micrograph of the mucosa of the ileum of Labeo niloticus showing the apical parts of the enterocytes, microvilli, capitulum, terminal web, endocytotic channel, smooth endoplasmic reticulum, junctional complex, lysosomes and mitochondria.

Fig. (33): Electron micrograph of the enterocytes of the ileum of Labeo niloticus showing the chylomicrons, Golgi apparatus, rough endoplasmic reticulum, free ribosomes microtubules, lateral cell membrane and nucleus.

Fig. (34): Electron micrograph of the basal parts of the enterocytes of the ileum of Labeo niloticus showing the mitochondria, basal cell membrane and basement membrane.

Fig. (35): Electron micrograph of the enterocytes of the ileum of Labeo niloticus showing the nucleus, euchromatin, heterochromatin, nuclear envelope, nuclear pore, rough endoplasmic reticulum and microtubules.

Fig. (36): Electron micrograph of the rectal mucosa of Labeo niloticus showing the enterocytes, microvilli, junctional complexes, mitochondria and lysosomes.

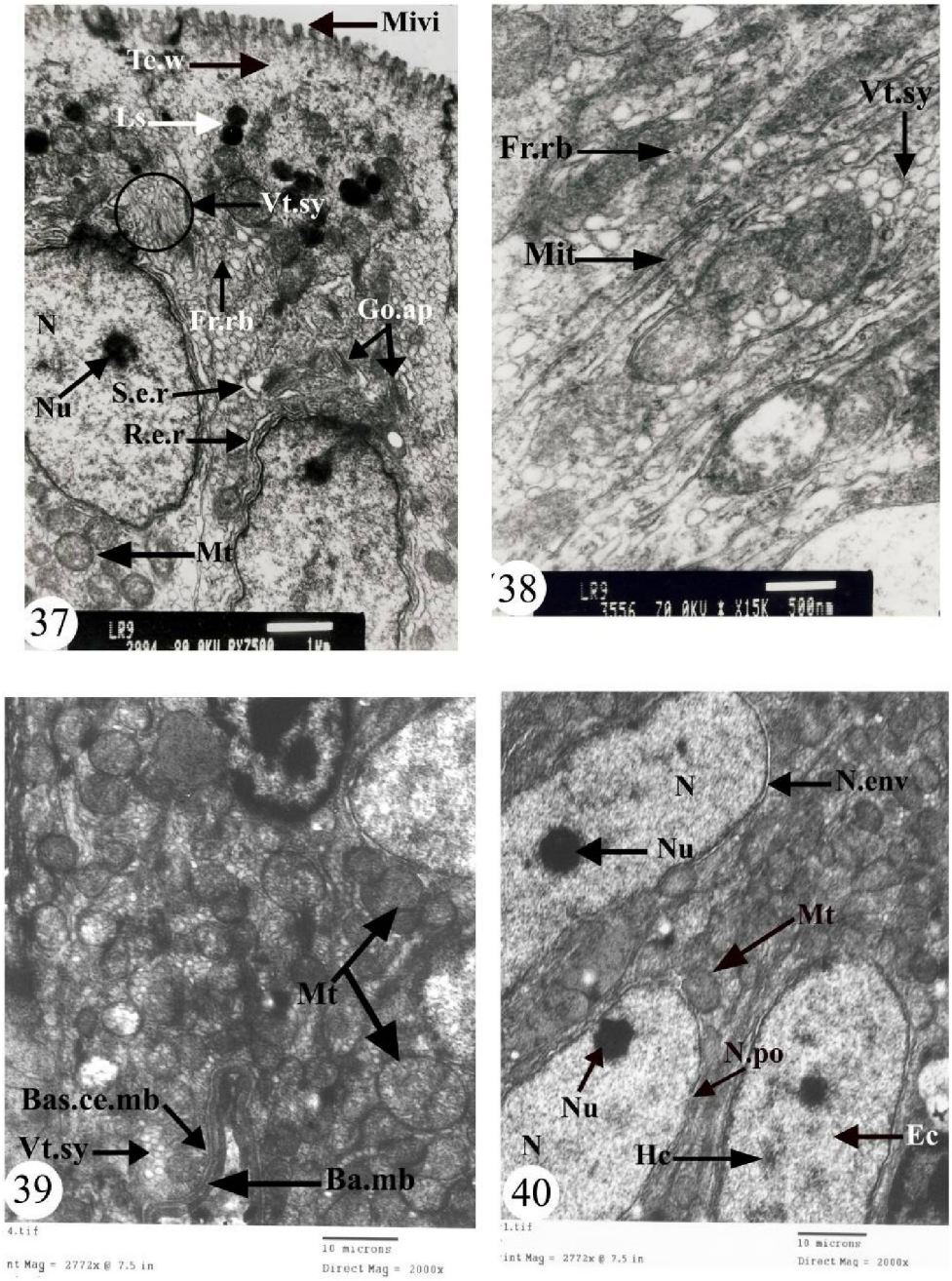

Fig. (37): Electron micrograph of the rectal enterocytes of Labeo niloticus showing the microvilli, terminal web, Golgi apparatus, rough and smooth endoplasmic reticula, free ribosomes, vesiculotubular system, mitochondria, lysosomes, nucleus and nucleolus.

Fig. (38): Electron micrograph of the rectal enterocytes of Labeo niloticus showing the free ribosomes, microtubules and vesiculotubular system.

Fig. (39): Electron micrograph of the rectal enterocytes of Labeo niloticus showing the mitochondria, vesiculotubular system, basal cell membrane and basement membrane.

Fig. (40): Electron micrograph of the rectal enterocytes of Labeo niloticus showing the nuclei, nucleoli, euchromatin, heterochromatin, nuclear envelopes, nuclear pore and mitochondria.

LIST OF ABBREVIATIONS

Ba.mb	Basement membrane.
Bas.ce.mb	Basal cell membrane.
Bl.v	Blood vessel.
Br.bo	Brush border.
C.m.lr	Circular muscle layer.
Cap	Capitulum.
Chy	Chylomicrons.
Ec	Euchromatin.
Enc.ch	Endocytotic channel
Enc.ves	Endocytotic vesicles.
Ent	Enterocyte.
Fr.rb	Free ribosomes.
G.ce	Goblet cell.
Go.ap	Golgi apparatus.
Hc	Heterochromatin.
Jn.comp	Junctional complex.
L.m.lr	Longitudinal muscle layer.
La.pr	Lamina propria.
Lat.ce.mb	Lateral cell membrane.
Lp.dr	Lipid droplet.
Ls	Lysosomes.
Ly	Lymphocyte.
Mifi	Microfilaments.
Mit	Microtubules.
Mivi	Microvilli.
Mt	Mitochondria.
Muc	Mucosa.
Muc.ep.ce	Mucosal epithelial cells.
Mus	Muscularis.
N	Nucleus.
N.env	Nuclear envelope.
N.po	Nuclear pore.
Nu	Nucleolus.
R.e.r	Rough endoplasmic reticulum.
S.e.r	Smooth endoplasmic reticulum.
Ser	Serosa.
Sm	Submucosa.
Te.w	Terminal web.
Vi	Villi.
Vt.sy	Vesiculotubular system.

4. Discussion

The observations obtained from the present study indicate that the relative gut length of the herbivorous *Labeo niloticus* is longer than that of the carnivorous *Schilbe mystus*. These results agree with those of Grau *et al.* (1992), in *Seriola dumerili*, Petrinec *et al.* (2005), in *Esox lucius* and *Silurus glanis,* and Carrassón *et al*. (2006), in *Dentex dentex*, who stated that these carnivorous species possess a short gut. In addition, Elliott and Bellwood (2003) postulated that the corallivores, omnivores and herbivores possess the longest intestines, but the carnivores possess the shortest intestines. This concept was also postulated by German and Horn (2006), in the herbivorous *Cebidichthys violaceus* and the carnivorous *Anoplarchus purpurescens*, and Horn *et al.* (2006), in the herbivorous estuarine *Atherinopsis affinis* and the carnivorous *Atherinopsis californiensis* and *Leuresthes tenuis.*

Due to the lower digestive efficiency, the herbivorous *Labeo niloticus* is characterized by an extremely long gut to maximize the assimilation of the nutrients. This functional explanation agrees with that of Albrecht *et al.* (2001) in *Leporinus friderici* and *Leporinus taeniofasciatus*, who suggested that the long intestine of the herbivorous species is due to some components of the diet, which are slowly digested and require both a longer time and more extensive enzymatic exposure.

In *Schilbe mystus*, there is a prominent constriction, the pyloric sphincter, between the pyloric portion of the stomach and the intestine, which may serve to control the food passage into the duodenum. This result agrees with that of Weinreb and Bilstad (1955), in *Salmo gairdneri irideus*, Martin and Blaber (1984), in three *Ambassis* species, Ostos Garrido *et al.* (1996), in *Anguilla anguilla,* Albrecht *et al.* (2001), in *Leporinus friderici* and *Leporinus taeniofasciatus*, and Mai *et al.* (2005), in *Pseudosciaena crocea*.

The intestinal muscularis of both studied species is differentiated into two layers of unstriated muscle fibres: an inner circular layer and an outer longitudinal one. This finding agrees with that of Gargiulo *et al.* (1998), in *Tilapia* spp., and Albrecht *et al.* (2001), in *Leporinus friderici* and *Leporinus taeniofasciatus*. However, this result contradicts with that of Grau *et al.* (1992) who described three layers of unstriated muscle fibres throughout the whole intestinal length of *Seriola dumerili*.

The intestinal mucosa of both studied species is built up of two types of cells: the enterocytes and goblet cells. This result agrees with that of Cataldi *et al.* (1987), in *Sparus aurata*, and Gargiulo *et al.* (1998), in *Tilapia* spp. The enterocytes of the intestine of both studied species are tall columnar cells with numerous apical microvilli, forming the brush border, and are interconnected in the apical regions by the junctional complexes. These results agree with those of Olsen *et al*. (1999) in *Salvelinus alpinus*. Moreover, Kiliaan *et al*. (1996) suggested that the junctional complexes of the enterocytes of *Carassius auratus* may prevent the paracellular leak of ions and macromolecules. In addition, Zambonino Infante and Cahu (2001) reported that the intestinal brush border membranes of some marine teleosts are linked to enzymes, such as peptidases and disaccharidases, which lead to the

total digestion of the diet components and allowing their absorption by the enterocytes.

The height of the enterocyte microvilli, in both studied species, decreases from the anterior to the posterior region of the intestine. This result agrees with that of Kuperman and Kuz'mina (1994), in *Esox lucius* and *Lota lota*, who stated that the height of the microvilli is certainly dependent on the localization of the enterocytes in the different regions of the fish intestine. In addition, they reported that the maximal height of the enterocyte microvilli of these fishes is observed in the anterior region of the intestine.

The tips of some microvilli of the enterocytes of the anterior part of the intestine of both studied species possess blebs or capitula, which may increase the apical surface and intensify penetration. Similar results were reported by Kuperman and Kuz'mina (1994) in *Esox lucius, Lota lota* and *Abramis brama*.

The enterocytes of the anterior portion of the intestine of *Schilbe mystus* contain numerous large lipid droplets, while those of *Labeo niloticus* contain small chylomicron particles. These observations suggest that the enterocytes of the anterior portion of the intestine are involved in lipid absorption. This suggestion is in accordance with that of Stroband and Debets (1978), in *Ctenopharyngodon idella,* Anderson (1986), in *Girella tricuspidata*, Deplano *et al*. (1989), in *Dicentrarchus labrax,* Olsen *et al*. (1999), in *Salvelinus alpinus,* Gallagher *et al.* (2001), in *Lagodon* rhomboids, Caballero *et al*. (2003), in *Sparus aurata,* Kozarić *et al*. (2004), in *Merluccius merluccius,* and Carrassón *et al*. (2006), in *Dentex dentex*. Moreover, Fontagné *et al.* (1998), in *Cyprinus carpio*, stated that the intestinal fat droplets can be interpreted as a temporary storage form of the re-esterified fatty acids.

The enterocytes of the duodenum and ileum of *Schilbe mystus* have no endocytotic vesicles, while the enterocytes of the intestinal swelling and ileum of *Labeo niloticus* contain a few numbers of endocytotic channels. Noaillac-Depeyre and Gas (1974 and 1976), in *Cyprinus carpio* and *Tinca tinca*, respectively, and Olsen *et al*. (1999), in *Salvelinus alpinus*, suggested that the endocytosis of lipid is not a major route of lipid absorption in the anterior intestine enterocytes. Moreover, Stroband and Debets (1978) stated that the anterior and middle segments of the intestine of the herbivorous *Ctenopharyngodon idella* possess endocytotic vesicles to uptake protein macromolecules.

The rectal mucosa of the carnivorous *Schilbe mystus* possesses numerous goblet cells amongst the enterocytes, while that of the herbivorous *Labeo niloticus* possesses a few numbers of goblet cells. numerous mucosal goblet cells were also found by Martin and Blaber (1984), in three carnivorous *Ambassis* species, and Grau *et al.* (1992), in the carnivorous *Seriola dumerili*. The farmer authors added that the mucus may serve as a lubrication aid to defecation. On the other hand, a few numbers of mucosal goblet cells were also reported by Girgis (1952) in the herbivorous *Labeo horie.*

The rectal muscularis of both studied species is thicker than that of the rest of the intestine. This result agrees with that of Grau *et al.* (1992) in *Seriola dumerili*, but contradicts with that of Gargiulo *et al.* (1998) who stated that the thickness of the rectal muscularis of *Tilapia* spp. is the same as the rest of the intestine.

The enterocytes of the rectum of *Schilbe mystus* possess endocytotic vesicles in the terminal web. This observation agrees with that of Deplano *et al*. (1989), in *Dicentrarchus labrax,* Olsen *et al*. (1999), in *Salvelinus alpinus,* Kozarić *et al*. (2004), in *Merluccius merluccius,* and Olsen *et al*. (2005), in *Oncorhynchus mykiss*, who stated that the presence of the endocytotic vesicles in the rectal enterocytes may be a complement to the luminal protein digestion by absorbing protein macromolecules. Moreover, Gargiulo *et al.* (1998), in *Tilapia* spp, suggested that the endocytosis is followed by the intracellular digestion by the lysosomes.

The rectal enterocytes of *Labeo niloticus* possess an extensive and a well-developed vesiculotubular system, which may be involved in ion transport. This observation agrees with that of Pack *et al.* (1996) in the stomachless *Brachydanio rerio*. In addition, the rectal enterocytes of *Labeo niloticus* have no prominent endocytotic vesicles. Stroband and Debets (1978) reported the same observation in the posterior segment of the intestine of the herbivorous *Ctenopharyngodon idella*.

The mucosa of the whole intestine of both studied species contains numerous lymphocytes, which may play a role in protecting the fish from pathogenic organisms. This result agrees with that of Osman and Caceci (1991), in *Tilapia nilotica,* and Park and Kim (2001), in *Misgurnus mizolepis.* Moreover, Temkin and McMillan (1986) stated that the gut of *Carassius auratus* lacks the discrete aggregations of lymphoid tissue, such as the Peyer's patches of mammals, but possesses a diffused lymphoid tissue as numerous lymphocytes.

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Fuzzy Ideals in CI-algebras

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Abstract: The fuzzification of ideals in CI-algebras is considered, and several properties are investigated. Characterizations of a fuzzy ideal are provided. **Mathematical Subject Classification:** 06F35, 03G25, 08A30.
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Keywords: Transitive, Self distributive, fuzzy ideal, Upper set.

1. Introduction:

Y. Imai and K. Iséki introduced two classes of abstract algebras: BCK-algebras and BCI-algebras ([3, 4]). It is known that the class of BCK-algebras is a proper subclass of the class of BCI-algebras. In [1, 2], Q. P. Hu and X. Li introduced a wide class of abstract: BCH-algebras. They have shown that the class of BCI-algebras is a proper subclass of the class of BCH-algebras. In [6], B. L. Meng introduced the notion of a CI-algebra as a generation of a BE-algebra. In this paper, we consider the fuzzification of ideals in CI-algebras. We introduce the notion of fuzzy ideals in CI-algebra, and investigate related properties. We give characterizations of a fuzzy ideal in CI-algebras.

2 Preliminaries

Definition 2.1 [6]:

An algebraic system $(X,*,1)$ of type (2, 0) is called a CI-algebra if it satisfying the following conditions:

(1) $x*x=1,$ (2.1)

(2) $1*x=x,$ (2.2)

(3) $x*(y*z)=y*(x*z)$

for all $x,y,z\in X$. (2.3)

We introduce a relation "$\leq$" on X by $x\leq y$ if and only if $x*y=1$ for all $x,y\in X$. (2.4)

Definition 2.2[5]:

A CI-algebra $(X,*,1)$ is said to be transitive if it satisfies:

$(y*z)*((x*y)*(x*z))=1$ for all $x,y,z\in X$. (2.5)

A CI-algebra $(X,*,1)$ is said to be self-distributive if it satisfies:

$x*(y*z)=(x*y)*(x*z)$ for all $x,y,z\in X$. (2.6)

Note that every self-distributive is transitive.

A non-empty subset S of an CI-algebra X is said to be a subalgebra of X if $x*y\in S$ whenever $x,y\in S$.

In an CI-algebra, the following identities are true:

(4) $y*((y*x)*x)=1.$ (2.7)

(5) $(x*1)*(y*1)=(x*y)*1.$ (2.8)

Definition 2.3:

A non empty subset I of a CI-algebra X is said to be a an ideal of X if it satisfies:

(I_1) If $x\in X$ and $a\in I$, then $x*a\in I$, i.e., $X*I\subseteq I$, (2.9)

(I_2) If $x\in X$ and $a,b\in I$, then $(a*(b*x))*x\in I$. (2.10)

Lemma 2.4 [5]:

Let X be a CI-algebra. then

(i) Every ideal of X contains 1,

(ii) If I is an ideal of X, then $(a*x)*x\in I$ for all $a\in I$ and $x\in X$.

3 Fuzzy ideals in CI-algebras

In what follows, let X denote a CI-algebra unless otherwise specified.

Definition 3.1:

A fuzzy set μ is called fuzzy ideal of X if it satisfies the following:

1) $\mu(x*y)\geq\mu(y)$, for all $x,y\in X$, (3.1)

2) $(\mu((x*(y*z))*z)\geq\min\{\mu(x),\mu(y)\})$, for all $x,y,z\in X$. (3.2)

Theorem 3.2:

Let μ be a fuzzy set in X. Then μ is a fuzzy ideal of X if and only if it satisfies:

$(\forall \alpha \in [0,1])(U(\mu;\alpha) \neq \phi \Rightarrow U(\mu;\alpha)$ is an ideal of X) (3.3)

where $U(\mu;\alpha) := \{x \in X \mid \mu(x) \geq \alpha\}$.

Proof. Assume that μ is a fuzzy ideal of X. Let $\alpha \in [0,1]$ be such that $U(\mu;\alpha) \neq \phi$. Let $x, y \in X$ be such that $y \in U(\mu;\alpha)$. Then $\mu(y) \geq \alpha$, and so $\mu(x * y) \geq \mu(y) \geq \alpha$ by (3.1). Thus $x * y \in U(\mu;\alpha)$. Let $x \in X$ and $a, b \in U(\mu;\alpha)$. Then $\mu(a) \geq \alpha$. It follows from (3.2) that

$$\mu((a * (b * x)) * x) \geq \min\{\mu(a), \mu(b)\} \geq \alpha$$

so that $(a * (b * x)) * x \in U(\mu;\alpha)$. Hence $U(\mu;\alpha)$ is an ideal of X.

Conversely, suppose that μ satisfies (3.3). If $\mu(a * b) < \mu(b)$ for some $a, b \in X$, then $\mu(a * b) < \alpha_0 < \mu(b)$ by taking $\alpha_0 := \frac{1}{2}(\mu(a * b) + \mu(b))$. Hence $a * b \notin U(\mu;\alpha_0)$ and $b \in U(\mu;\alpha_0)$, which is a contradiction. Let $a, b, c \in X$ be such that $\mu((a * (b * c)) * c) < \min\{\mu(a), \mu(b)\}$. Taking

$$\beta_0 := \frac{1}{2}(\mu((a * (b * c)) * c) + \min\{\mu(a), \mu(b)\}$$

, we have $\beta_0 \in [0,1]$ and $\mu((a * (b * c)) * c) < \beta_0 < \min\{\mu(a), \mu(b)\}$. It follows that $a, b \in U(\mu;\beta_0)$ and $(a * (b * c)) * c \notin U(\mu;\beta_0)$. This is a contradiction, and therefore μ is a fuzzy ideal of X.

Lemma 3.3:

Every fuzzy ideal μ of X satisfies the following inequality:

$\mu(1) \geq \mu(x)$, for all $x \in X$. (3.4)

Proof. Using (2.1) and (3.1), we have

$\mu(1) = \mu(x * x) \geq \mu(x)$, for all $x \in X$

Example 3.4:

Let $X = \{1, a, b, c, d, 0\}$ be a set with the following Cayley table:

*	1	a	b	c	d	0
1	1	a	b	c	d	0
a	1	1	a	c	c	d
b	1	1	1	c	c	c
c	1	a	b	1	a	b
d	1	1	a	1	1	a
0	1	1	1	1	1	1

Then $(X, *, 1)$ is a CI-algebra.

(1) Let μ be a fuzzy set in X defined by

$$\mu(x) := \begin{cases} 0.7 & \text{if } x \in \{1, a, b\}, \\ 0.2 & \text{if } x \in \{c, d, 0\}. \end{cases}$$

Then

$$U\{\mu;\alpha) = \begin{cases} \phi & \text{if } \alpha \in (0.7, 1], \\ \{1, a, b\} & \text{if } \alpha \in (0.2, 0.7], \\ X & \text{if } \alpha \in [0, 0.2]. \end{cases}$$

Note that $\{1, a, b\}$ and X are ideals of X, and so μ is a fuzzy ideal of X.

(2) Let ψ be a fuzzy set in X defined by

$$\psi(x) := \begin{cases} 0.6 & \text{if } x \in \{1, a\}, \\ 0.4 & \text{if } x \in \{b, c, d, 0\}. \end{cases}$$

Then

$$U\{\psi;\beta) = \begin{cases} \phi & \text{if } \beta \in (0.6, 1], \\ \{1, a\} & \text{if } \beta \in (0.4, 0.6], \\ X & \text{if } \beta \in [0, 0.4]. \end{cases}$$

Note that $\{1, a\}$ is not an ideal of X since

$(a * (a * b)) * b = (a * a) * b = 1 * b = b \notin \{1, a\}$

.

Hence ψ is not a fuzzy ideal in X.

Proposition 3.5:

If μ is a fuzzy ideal of X, then

$(\forall x, y \in X)(\mu((x * y) * y) \geq \mu(x))$. (3.5)

Proof. Taking $y = 1$ and $z = y$ in (3.2) and using (2.2) and lemma 3.3, we get

$\mu((x*y)*y) = \mu((x*(1*y))*y) \geq \min\{\mu(x), \mu(1)\} = \mu(x)$

for all $x, y \in X$.

Corollary 3.6:

Every fuzzy ideal μ of X is order preserving, that is, μ satisfies:

$$(\forall x, y \in X)\ (x \le y \Rightarrow \mu(x) \le \mu(y)). \quad (3.6)$$

Proof. Let $x, y \in X$ be such that $x \le y$. Then $x * y = 1$, and so

$$\mu(y) = \mu(1 * y) = \mu((x * y) * y) \ge \mu(x)$$

by (2.2) and (3.5).

Proposition 3.7:

Let μ be a fuzzy set in X which satisfies (3.4) and

$$(\forall x, y, z \in X)\ (\mu(x * z) \ge \min\{\mu(x * (y * z)), \mu(y)\}). \quad (3.7)$$

Then μ is order preserving.

Proof. Let $x, y \in X$ be such that $x \le y$. Then $x * y = 1$, and so

$$\mu(y) = \mu(1 * y) \ge \min\{\mu((1 * (x * y)), \mu(x)\} = \min\{\mu(1 * 1), \mu(x)\} = \mu(x)$$

by (2.1), (2.2), (3.7) and (3.4).

We give a characterization of fuzzy ideals.

Theorem 3.8:

Let X be a transitive CI-algebra. A fuzzy set μ in X is a fuzzy ideal of X if it satisfies condition (3.4) and (3.7).

Proof. Assume that μ is a fuzzy ideal of X. By lemma 3.3, μ satisfies (3.4). Since X is transitive, we have

$$(y * z) * z \le (x * (y * z)) * (x * z) \quad (3.8)$$

i.e., $((y * z) * z) * ((x * (y * z)) * (x * z)) = 1$

for all $x, y, z \in X$. It follows from (2.2), (3.2) and proposition 3.5 that

$$\begin{aligned} \mu(x * z) &= \mu(1 * (x * z)) \\ &= \mu((((y * z) * z) * ((x * (y * z)) * (x * z))) * (x * z)) \\ &\ge \min\{\mu((y * z) * z), \mu(x * (y * z))\} \\ &\ge \min\{\mu(x * (y * z)), \mu(y)\}. \end{aligned}$$

Hence μ satisfies (3.7).

Corollary 3.9:

Let X be a self-distributive CI-algebra. A fuzzy set μ in X is a fuzzy ideal of X if it satisfies condition (3.4) and (3.7).

Proof. Straightforward.

For every $a, b \in X$, let μ_a^b be a fuzzy set in X defined by

$$\mu_a^b(x) := \begin{cases} \alpha & \text{if } a * (b * x) = 1, \\ \beta & \text{otherwise} \end{cases}$$

for all $x \in X$ and $\alpha, \beta \in [0,1]$ with $\alpha > \beta$.

The following example shows that there exist $a, b \in X$ such that μ_a^b is not a fuzzy ideal of X.

Example 3.10:

Let X = {1, a, b, c, d, 0} be a CI-algebra as in Example 3.4. Then μ_1^b is not a fuzzy ideal of X since

$$\begin{aligned} \mu_1^a((a * (a * b)) * b) &= \mu_1^a((a * a) * b) = \mu_1^a(1 * b) \\ &= \mu_1^a(b) = \beta < \alpha = \mu_1^a(a) \\ &= \min\{\mu_1^a(a), \mu_1^a(a)\}. \end{aligned}$$

Lemma 3.11:

A nonempty subset I of X is an ideal of X if it satisfies

$$1 \in I, \quad (3.9)$$

$$(\forall x, z \in X)(\forall y \in I)(x * (y * z) \in I \Rightarrow x * z \in I). \quad (3.10)$$

Proof. Let I be an ideal of X. Using (2.1) and (2.9), we have $1 = a * a \in I$ for all $a \in I$. We prove the following assertion:

$$(\forall x \in I)(\forall y \in X)(x * y \in I \Rightarrow y \in I). \quad (3.11)$$

Let $x \in I$ and $y \in X$ be such that $x * y \in I$. Then

$$y = 1 * y = ((x * y) * (x * y)) * y \in I$$

by (2.10). Now, let $x, z \in X$ and $y \in I$ be such that $x * (y * z) \in I$. Then $y * (x * z) \in I$ by (2.3). Since $y \in I$, it follows from (3.11) that $x * z \in I$. Hence (3.10) is valid.

Let X be an CI-algebra and $a, b \in X$. Define $A(a,b)$ by

$$A(a,b) = \{x \in X \mid a * (b * x) = 1\}.$$

We call $A(a,b)$ an upper set of a and b. It is easy to see that $1, a, b \in A(a,b)$ for all $a, b \in X$.

Theorem 3.12:

Let μ be a fuzzy set in X. Then μ is a fuzzy ideal of X if and only if μ satisfies the following assertion:

$$(\forall a,b\in X)(\forall \alpha\in[0,1])(a,b\in U(\mu,\alpha)\Rightarrow A(a,b)\subseteq U(\mu,\alpha)). \quad (3.12)$$

Proof. Assume that μ is a fuzzy ideal of X and let $a,b\in U(\mu;\alpha)$. Then $\mu(a)\geq\alpha$ and $\mu(b)\geq\alpha$. Let $x\in A(a,b)$. Then $a*(b*x)=1$. Hence $\mu(x)=\mu(1*x)=\mu((a*(b*x))*x)\geq\min\{\mu(a),\mu(a)\}\geq\alpha$, and so $x\in U(\mu;\alpha)$. Thus $A(a,b)\subseteq U(\mu;\alpha)$. Conversely, suppose that μ satisfies (3.12). Note that $1\in A(a,b)\subseteq U(\mu;\alpha)$ for all $a,b\in X$. Let $x,y,z\in X$ be such that $x*(y*z)\in U(\mu;\alpha)$ and $y\in U(\mu;\alpha)$. Since $(x*(y*z))*(y*(x*z))=(x*(y*z))*(x*(y*z))=1$ by (2.3) and (2.1), we have $x*z\in A(x*(y*z),y)\subseteq U(\mu;\alpha)$. It follows from Lemma 3.11 that $U(\mu;\alpha)$ is an ideal of X. Hence μ is a fuzzy ideal of X by Theorem 3.2.

Corollary 3.13:

If μ is a fuzzy ideal of X, then

$$(\forall \alpha\in[0,1])(U(\mu,\alpha)\neq\phi\Rightarrow U(\mu,\alpha)=\bigcup_{a,b\in U(\mu,\alpha)}A(a,b)). \quad (3.12)$$

Proof. Let $\alpha\in[0,1]$ be such that $U(\mu;\alpha)\neq\phi$. Since $1\in U(\mu;\alpha)$, we have

$$U(\mu;\alpha)\subseteq\bigcup_{a\in U(\mu;\alpha)}A(a,1)\subseteq\bigcup_{a,b\in U(\mu;\alpha)}A(a,b).$$

Now, let $x\in\bigcup_{a,b\in U(\mu;\alpha)}A(a,b)$. Then their exist $\nu,\lambda\in U(\mu;\alpha)$ such that $x\in A(\nu,\lambda)\subseteq U(\mu;\alpha)$ by Theorem 3.12. Thus $\bigcup_{a,b\in U(\mu;\alpha)}A(a,b)\subseteq U(\mu;\alpha)$.

This completes the proof.

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Investigating the Role of Total Quality Management Eminence Pattern in Strategic Evolution of Organizations

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Abstract: This research has been performed in purpose of definition and explanation of Total quality management advanced model and investigating its role in organizations' strategic evolution. This article is in type of developmental researches and its performance method is survey and library studies. Moreover information collecting tool is taking notes. In literature of subject of this article initially the principles of total quality management has been discussed, so that we can obtain better perception in order to applying this system in business and improvement of organizations' performance. In order to apply total quality management in organizations strategically, we have examined TQM movement in Japan and some cases about using of this method have been mentioned. In fact, in this article the model which has been named Total Quality Management Advanced Model (TQMEX) is based on TQM facts and has been modeled according to instructions of this model. A referendum which has been done in Japan, Hong Kong and South Korea indicates the importance of Japanese system of total quality management advanced model in order to implement total quality management system in organizations. Finally, results and information obtained from this research provide criteria and methods for which are the result of the large organizations' experiences that not only have passed two world's oil crisis and Asian financial crisis successfully, but also have continued their growth while these two crises.
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Keywords: Total quality management, change management, strategic evolution, financial crisis

1. Introduction

The challenge resulted from global competition has led to increase the pressure on organizations in order to improve abilities, skills and quality of products and services. The principles of total quality management (TQM) has been accepted broadly at global level as a device for improvement of organizational performance, organizations' nature and the manner of encounter market's special challenges.

Asian financial crisis which occurred in the middle of 1997 and was began from cash-financial crisis in Thailand, resulted in beginning a chain movement which impressed all Asian countries.

This crisis and created paradoxical changes learned many lessons in order to improve economic and developmental situation in organizations and different Asian countries' industry which has led to widespread changes in these organizations' structure.

These changes have had competent effect on total quality management movement. Total quality management principles has been accepted broadly at global level as a device for improvement of organizational performance, organizations' nature and the manner of encounter market's special challenges.

2. Concept of Total Quality Management (TQM)

In 1990s, total quality management became one of the most attractive competition strategies for organizations which were exploring significant difference with others.

Academicians and industrialists emphasized this attraction which encourages organization total quality management to focus on customer's needs by improvement process and respect to costs improvement, quality and customer satisfaction.

Total quality management was based on active tracking continuous improvement, perception of organization's internal customers' attitude, training and development in all organizational dimensions.

But some also believed that total quality management philosophy has its limitations. Sitkin's findings show that total quality management is dangerous for discussions such as oversell and its implementation is damaging and this is a part of total quality management inability.

Researchers believe that nowadays total quality management marketing has changed to a special industry and perception of total quality management has been expanded as a result of its multilateral performance.

Luthauns believes that total quality management is not able to perceive future evolutions and suggests that enough time spend on total quality management.

A group also believes that learner organization, because of its interdependence, is the next logical

step in changes' valuation. (Baran-Doust and Rahmani, 2003)

In Japanese industry total quality management has led to wonderful results.

But what does have converted TQM to a necessity? In fact total quality management (TQM) is a kind of total integrity in all over the organization in order to obtain competitive advantage through continuous improvement in all organization's activities.

If we intend to interpret each word in "total quality management", we can say:

- **Quality:** comply with all customers' needs and their implied requirements.
- **Management**: An executive board which completely has became as committee and coordinate.
- Ideally all individuals within the organization should be organized and classified. Based on Doming's researches in 1986, about 94% of problems are related to management and systems which are created through them.

Thus, managers' commitment should be done before frontline workers. Total quality management requires that all individuals within organization including frontline workers involve the process.

Thus, the mentioned definition about total quality management indicates a balance between the real meaning of the word and its ideal state.

Despite years of educational experience in the field of total quality management and leading more than 30 research cases in contact with more than 1000 subjects related to total quality management, the result of experiences and educations has been mentioned in this article.

The obtained results indicate that on average less than 20% of cases have obtained desired result. In fact when I implemented total quality management for the first time, I was in that category of 80%.

I believe that the real value of this method and process in this subject is that this system shows us that in these failures, workers were not guilty, but rather managers are responsible for these failures.

What should do managers for implementing programs and providing suitable devices such as controller of words syllables.

In this research we re-examine Dr. Deming's findings in 1986. In fact in 1993 before death of W.E. Deming, he, in his previous book: "Modern Economy" has changed the effect of structure from 94% to 97%.

If this is true, then how can organizations develop and improve their competent managers?

The answer to this question is in managers' continuous learning which is done in learner organizations. For example Ford suggests that total quality management provide necessary environment in order to create learner organization.

Total quality management success is related to learning ability, attraction, conformity and performing organization's attitude change and combining them in the organization. For example Barrow admits that this relationship appears in two ways. First: it's a cause and effect relationship. Learner organization is the result of performing total quality management in the organization. Second: there is strong correlation between these two systems, process improvement and organizational learning which are performing in a simultaneous and integral manner.

Garvin explains that if total quality management is done as a philosophy, then a series of techniques can be a cycle for organization's learning. (Baran-Doust and Rahmani, 2003).

3. Organizational Evolution Process:

An old proverb says that the only thing that remains constant in the world is evolution. If evolution be as a part of our daily life, then how can we make it under control rather than be controlled by it. When evolution becomes a longtime process in the organization, this will lead to evolution in organization's culture.

An obvious example of this situation is learner organization.

When individuals within the organization are motivated to present new ideas and identify failings, these two subjects will be of very important factors in organization's success.

Let's go back to step and look at traditional strategic evolution processes which can summarize it as 5 steps:

1. Purpose, 2. Mission, 3. Behavior, 4. Culture.

But in modern model and method, this process will be as follow:

1. Operation, 2. Behavior, 3.Mission, 4. Imagination and purpose, 5. Culture.

In fact, the first step is not a new thing. Formerly, Pitter and Waterman (1982) based on researches and findings obtained from 46 successful companies indicated that most of them have selected Operation as the first step so that they can obtain business improvement through it.

2. The new theory suggests that it's only Operation which leads to staff's behavioral evolution. (R. H. Waterman and T. J. Peters, 1982).

This is caused by organizational learning process.

In this direction Reg Warrancy states that there is no learning without operation and no operation without learning.

If the learning processes are done successfully in organization, then organizational behavior leads to a dynamic and challenging state. Finally this will change top managers' perception about organization' mission. Thus, those will be successful who are aware of organization's mission and step toward better behavior.

When top managers in the organizations perform organizational mission in a better manner, this shifts organization to new definition of global competition.

As soon as a new structure is formed in the organization, this will result in creating new purpose in organization which finally leads to new culture within the organization. One of the best examples of creating new culture in the organization is Kaisen model which in fact is a Japanese word and means continuous improvement. This new model has many successful types. Many successful American and Japanese companies have emphasized on the subject of completely open workspace in the organization.

Of very common examples are completely open official processes in official environments and factory design. In Malaysia, companies such as Sony-Hewlet Packard hold their top managers' meetings completely open so that through which they can transfer organizational purposes to all organizational and sale levels, directly. Mc Donald restaurants have begun to build completely open kitchens so that even passers can observe production process directly.

4. Quality Management's Strategic Evolution:

Always, evolutions in the organizations don't lead to success.

In fact if evolutions don't direct correctly, this will be similar to Titanic ship which try to prevent collision with icebergs but final result is tragic. In other words, if evolution doesn't direct and organized effectively, then the better choice is that: evolution is that we don't change! For example many see engineers believe that if Titanic ship in its front part had appropriate measures against icebergs through appropriate design, then never was drowned.

Mr. Rekner in a conference in 1995 has predicted the future of this subject as following:

- By entering multinational and social markets, the quality competition will be intense.
- There will be created many requests for suppliers.
- ISO9000 will embraced all over the world..
- Awarding prizes such as European quality prize (Baldrige) cause motivation of companies and this will lead to quality expansion in all over the world.

Jouran's predictions have been ascertained and will be continued for years, specialty during two past years. ISO9000 standard series have been expanded like an explosion in all over the world and also have become a known fact. If we assume the year 1990 as a base, then the number of registered companies which have used quality standards, have had 100% growth. In late 1997, in a survey which was done by Mobil Company, it was specified that about 200000 companies around the world have received ISO9000 standard. Some of these companies such as Telecom British have tens of thousands of employees (Samuel K.M.Ho., 1999). For what reason quality standard owners receive this license. Some of the most important quality standard licenses and prizes are as follows:

- Deming Prize
- Quality Certificate of US (Quality Award "MBNQA" USA Malcolm Baldrige National

Deming prizes are received by Japanese companies except following cases:

- Taiwan Tube (Philips)
- Florida Light Power
- Division Lucent Technology (Power).

European quality licenses and American quality license also are two very strong models. If your country is none of these countries or doesn't have any bond with them, then you can choose one of these models as your organization's total quality management structure. Or use your country's total quality management system.

Very likely, the type of system you use is dependent on your company's geographic situation or its origin. If your company is placed in United States or has strong American origins, then the system you choose and is the more suitable one, is in type of MBNQA.

Both MBNQA and EQA models are comparable with respect to scoring and have similar scoring and scores in more than 1000 scores. In final making decision, determinant factor is probably organization's geographic situation which determines quality standard type. After selecting desired model, it's turn to provide required equipment in order to implement quality model (Samuel K. M. Ho., 1999).

Total quality management system is very similar to ISO9000. Independent of quality management system implementation, TQM must consider quality improvement and customers' factor. (Such as ISO9004.4 Standard)

A very suitable method in order to implement TQM is in fact following the example of companies which

have implemented this system successfully. UK pioneered implementing ISO9000 in Europe and this led to beginning a very large movement in Europe.

British quality organization and institute as the leader of this issue in Europe resulted in EQA model expansion in Europe broadly and many companies use this model for their assessment.
In fact the purpose of this CEO is that encourage companies in UK to use this model. Mr. Frank Malcolm believes that using this method results in creating evolutions which lead to organizational improvement.
Despite ISO9000 model, self-assessment models such as mentioned 3 models tell organizations what to do. Moreover mentioned models have ability to say people what performance they should have and how to do it and support it. Thus, in order to obtain mentioned quality methods we need a proved model.

5. Total Quality Management Advanced Model

In order to have a total and systematic method to implement total quality management, we should initially develop a conceptual model. This model should be simple, logical and comprehensive enough.
During the last post I had in foreigner countries, as the quality affairs specialist I was responsible for quality system designing in Malaysia during a 5 year program in order to apply in industry.
In consequence of the results obtained from this mission and also previous experiences and the researches I had about the best total quality management implementation performance, I presented the following total quality management model as the best method in order to implement total quality management.
This idea includes step by step processes which have the ability to implement in all over the world and finally it leads to implement total quality management in best possible form which has been presented in figure 1. (Ho. 1995)
The advanced total quality management model uses an integrated and coordinated method in order to support total quality management system. In fact this management system is a continuous improvement process which engages organization to be based on management quality. This model includes elements which are based on explaining TQM philosophy and implement it at all levels of the organization. The elements of TQM-9000 TQMEX ISO model which has been mentioned before will be explained more.

Japanese 5S Model

In fact 5S is a technique in order to create and protect a healthy work environment with high quality in the organization. This word, in fact indicates 5 Japanese words which English Meanings and specific samples have been presented in table 1.

6. Specific Examples of English Meaning of Japanese Word

Structurize Seiri: to organize, discarding worthless things.
Systematize Seiton: to methodize, retrieving a document in 30 seconds.
Sanitize Seiso: to make healthy, personal responsibility of cleaning.
Standardize Seiketsu: to standardize, transparency in saving data.
Self-discipline Shitsuke: creating discipline, 5S daily completion.
Table 1: 5S technique
This technique was used broadly in Japan. Most of individuals who have used this technique believe that not only this method is suitable for beauty and physical improvement of work environment, but also using it will be very useful for organization's processes improvement.
Apparently 5S techniques are applicable in all life's aspects. Many routine problems can be solved according to this technique. Unfortunately using this powerful method in order to improve quality is not known in west world. In order to implement this method simply we can use auditing and check lists provided by author (S.K, Ho 1995).
For example in using this method in Hong Kong based on previous successful instructions, recently Industry section of government has approved a plan. According to this plan about 2500 individuals of senior managers in country's industry are trained in this technique, while this plan's costs and budget are provided by government and project officer is the inventor of technique which should implement this project in country's industry during a 2 years plan.

7. Business and Organizational Processes Reengineering (BPR)

Homer and Champi has defined reengineering as following: re-thinking about organization's foundation and structure, re-designing organization's processes basically in order to obtain significant improvements which are accomplished critically and simultaneously from company's performances such as costs, quality, services and organization's performance rate.
In fact, reengineering make managers have a re-look at organization's traditional and old processes and engage them to focus on customers.
Many parent and leader companies in the world have obtained current situation through applying reengineering. Companies which have used

reengineering techniques have obtained significant results such as:

Improve relations with customer

- Decreasing life cycle in relation to market and marketing
- Increasing company's production ability
- Decreasing faults and defects
- Increasing company's profitability

In fact reengineering use a series of known and specific techniques in order to improve organizational outcomes and make more effective organization's traditional structure. This technique uses methods such as exact definition of work process, assessment and its accurate measurement, and reengineering in organization conflicts in order to improve customer's satisfaction and other different methods.

8. Quality Control Class (QCC)

Quality control circle in fact is a small group which includes organizational individuals and employees who work jointly so that help to cases such as organization's performance improvement, respect to personnel, creating work groups with high morale through development and improvement of group members' unlimited potential.

Japanese people experienced that about 95% of quality-related problems in many organizations can be solved through simple quality control methods such as 7quality control tools (QC7) (Inshikawa, 1986).

These 7 tools are:

- Parato diagram
- Information ranking
- Data registration form
- Histogram
- Transmittance diagram
- Control tables and diagrams

These tools help quality control class to hold brainstorming sessions systematically and analyze current issues critically and faster.

ISO9000

ISO9000 group and family include 7 standard types which are placed consequently. Out of these 7 standards, the only important and notable ones are: ISO 9003, ISO 9002, ISO 9001. In fact about 99% of registered ISOs in companies are in type of ISO, ISO 9001 and ISO 9002. 14 remained standards are only used as helper and guider.

Thus, the purpose of explaining this article is only to review more detailed ISO 9001 which was presented. ISO 9001 in fact is a quality system model which is used in order to guarantee quality in design, development, production, installation and services. This standard is a comprehensive model in quality systems presented by ISO.

9. Pure Production Maintain (TPM)

In 1972, Japanese board of maintaining JIPM Company defined this technique as one of the maintaining units.

In fact this system guarantees that all equipments and facilities in organization's each section such as design, production, construct and maintaining, work without any defect during their consumption life. Since the purpose of this technique is to increase the ability to product and operate equipment, the expression TPM has been known as pure production management (Sengu, 1992).

10. Validity of Advanced Quality Management Model

One of the important abilities of this method is that it can obtain total quality management through a step by step process. Moreover every these steps can be used exclusively and separately. Obtained results from each step also are assessed separately. This feature in fact is a great advantage. Because companies can select options which focus on their activities.

And also it's possible that if a company has not implemented a step, can go back to previous step.

In fact this model is a simple and flexible model. In order to prove that TQMEX-presented models are in type of efficient and perfect models and have proper quality and management aspects, Mr. Fang and me designed a questionnaire based on audits and examinations we had from Japanese, Hong Kong and UK companies.

Findings resulted from this research were published in HO and Fung magazine.

Analysis of results obtained from research, provided evidences which states existing differences and similarities in various companies in order to implement TQMEX. The total quality management issue is very important to all companies and this indicates that nowadays TQM is not only used in Japan but also is a global method.

Three main and final questions proposed in questionnaire, are:

1. Competency
2. Faults and defects
3. Possible improvements in their quality management system (including ISO 900 or based on TQM)

The results and issues presented by quality executive managers comprise important TQM-related information and suggestions that can be used by companies which tend to implement this system in their company.

a. NEC company
   - Competency and capability: to concentrate company's staff's consideration for strategic goals in competitive markets.
   - Fault and defect: it just provides necessary conditions to present to foreigner markets through ISO 9000 certificate.
   - Development and improvement: includes ultra-qualification concepts such as: safety, environment protection and so on. And expanding their goals to other dimensions and issues.

b. SUNYO Company
   - Competency and capability: creating consensus between executive directors. Spirit organization's members and improving corrective activities and efficiency in organization.
   - Faults and defects: organization's indirect sections need more conformity with TQM in a larger scale.
   - Improvement and development: emphasizing human factor in organization, leading by senior managers. Tendency to customer at all levels of organization.

c. Tokyo electricity distribution company:
- Competency and capability: staff was familiarized perfectly with concept of design-perform-audit-reaction. Quality control groups had great improvement.
- Faults and defects: organization's managers and respondents forgot improvement activities and consequently operation's form and appearance were considered rather that operation's origin.
- Improvements: led to creating and developing total quality management system technically. TQM should be executed by organization's managers, not by TQM counselors. This method should be explained completely to managers.

d. Nevita Motor Company:
   - Competency and capability: organization's all staff at each level of organization with respect to their responsibility type undertakes to follow the pattern of respect to customer. By using main and important methods in quality control, staff considered this system in order to improve the company.
   - Faults and defects: there was no defect while performance.
   - Improvements and developments: the concept of TQM in order to create a constant structure for organization under any conditions had continued permanently. Although naturally concepts which total quality management emphasize, should be changed with respect to variable conditions (according to environment changes).

For example respect to environment, organization's strategy about marketing or customer's satisfaction.

Evidences obtained from TQM performance in world's successful companies, obviously indicate that total quality management is like a trip through which organizations can go or get to anywhere. Managers' expectations from this system are creating large evolutions in organization with purpose of being better.

Thus we can simply understand that why these companies during two past crises (oil crisis and Asian financial crisis) not only have remained but also grown. In fact during 50 past years, these companies have laid their foundation based on TQM principles and built their business activities based on models presented by quality leaders such as Deming, Jouran and so on.

Despite some differences in methods which are used by these companies, they should always and continuously consider one case and have perfect ability with regard to it and that's organizational learning. Mr. Deming passed away in 90 but he still supports this concept that the only existing entertainment is learning.

11. Totaling and Conclusion

In this article concept of total quality management was re-investigated so that we can obtain a proper viewpoint in order to improve and promote business processes. The concept of total quality management requires systems integration and techniques which are used in quality.

Based on experiences which have been obtained from total quality management system performance in Japanese companies, we concluded that in order to implement total quality management we need a step by step process.

Both theoretical fields, i.e. personal experiences and results obtained from performed investigations, confirm that in order to implement total quality management we need to 5S systems implementation, reengineering, quality control circle, ISO 9000 and

pure production management which was named as advanced total quality management model.

Moreover in this article it was proved that the concept of advanced total quality management can be performed at all organizational levels and large organizations' senior managers. Consequently this model is suitable for companies which intend to be excellent. Obtained results are more useful for companies which intend to obtain organizations' and pioneer companies' business improvements in the world.

Moreover advanced total quality management model can be used as a system in order to access awards and quality certificates such as EQA. This model is a total mechanism which can be applied in order to create strategic evolutions in organization. By using of this model organizations become targeted to move toward business improvements and permanent and continuous development.

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Motivating Lecturers in Iran Teacher Education Centers

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Abstract: Motivated, healthy, happy, productive, knowledgeable and skillful lecturers increase efficiency in Teacher Education Centers. The main purpose of this research is to survey and represent methods of motivating lecturers. The goal of this study is to examine and to improve method of motivation. Also correlation and comparison are used to indicate rate of links and differences between study variables.The statistical society of this study was all teachers of Teacher Education Centers. Samples were selected non-randomly from each state, which 30% of this sample was examined randomly. Tools for this study were Mazlow's Hierarchy Questionnaires, Maslach Exhaust Questionnaire, and teachers' poll. An ordinal scale was used. The questionnaires were distributed in Teacher Education Centers by the executer et al. Also related documents were used. Descriptive and inferential analysis were used for data analysis. Regarding to five levels of Mazlow's Needs, average of 3.13 was obtained for motivation. This means that lecturers mostly desired "social belong." Average of motivation rate (exhaust rate) was 55.47 (31.69%). This means that lecturers exhaust rate is good with respect to maximum exhaust of 175 (100%). There is no difference for inferential analysis of data between male and female lecturers' view. Motivation level difference between men and women is meaningful, and difference of needs between skillful and unskilled lecturers is meaningful. There are correlations between age and record, age and education, age and exhaust, and record and education. Proposed strategies to improve lecturers' motivation are: 1. increment of salary (15.5%), 2. meritocracy (11%), and 3. appending Teacher Education Center to Ministry of Sciences, Researches, and Technology (10.8%).
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1. Introduction

Our country experienced many political, economical, and social problems during recent years, especially during 8 years of Holy Defense. Naturally, human resource was affected more than others. A significant part of these damages is observed in lecturers of Teacher Education. Respect to needs and motives have turned notice of researchers as a style to protect non-motivation, unability, and psychic stress, and hence exhaust and balance. Researches show that notice of needs and high readinesses have a large effect on removal of exhaust and preserve of psychic health. Improvement of psychic health of work environment is one of the tools to improve human resources in organizations. Motivated, healthy, creative and knowledgeable human resource must be used because it has an important effect to develop efficiency level of organization. Notice of psychic needs in educational environments is essential, and useful strategies must be used to improve motive and psychic needs of lecturers that tolerate most work load. Teacher Education Centers are subsidiaries of educational organizations to offer scientific-application trainings. The aim of this study is to investigate styles for improvement of motivation of lecturers of Iran Teacher Education Centers.

The results of studies show that psychic stresses are related to the following factors:

- Individual, organizational, and extra-organizational features: Those individual features that cause psychic stress are motive decrement, vocational dissatisfaction, teaching, role opposition, age, and weakness. Those organizational and extra-organizational features that cause psychic stress and exhaust are low time and much work, excess of students, less support by management, no participation of individuals in decision-making, and lack of a systematic strategy (Henri et.al;, 2003).
- Khalaji and Shafizadeh (2001) found the following in a research for relation of motivation and physical education and scientific knowledge of male sport teachers. Motivation is one of the most important areas of sport psychology and has an effective factor in teaching, exercise and rival. Analysis of data showed that there was no significant relation between motivation

and physical readiness and skills of male sport teachers, but there is a significant relation between motivation and scientific test [1].

- Musavi (2001) found the following results in his study, by a sample of 905 persons:
    1. Psychic stress of athletic staff was less than non-athletic ones in physical, behavioral, cognitive and exciting responses, but this difference was not significant between female teachers in $p<0.05$.
    2. Psychic stress diminished by increment of exercise sessions, but this difference was not significant between female teachers in $p<0.05$.
    3. Psychic stress of those staff with more than 20 years in athletic activities was less than other in $p<0.05$ in physical, cognitive and exciting responses.
- Henri et al (2003) showed that analysis method is used to recognized priority of effective factors on human resources according to AHP technique or hierarchical analysis process. Eight main components of this study are: motivation, organizational promise, power, organizational protect, job recognition, validity, performance feedback, and environmental compatibility, which motivation has the most share in staff performance [6].
- Sadeghi Borujerdi (2003) found the following results in his thesis:
  His society of 215 persons was under psychic stress and exhaust even they were in a desired level by psychic-physical measure. High psychic stress in members of scientific board, exhaust of PhD employees, anxiety in promissory employees, less psychic-corporal health of females than males, problems of management system, and status of scientific board, all were from those problems for which proposed strategies must be applied. This study showed that correlation between stressful job factors, exhaust, and psychic-corporal health is high and stress in each one can affect the others (Sadeghi Borujerdi, 2003).
- Sanei (2004) found the following results in his thesis:
    1. Regarding to statistical analysis of averages for physical readiness and exhaust, the results showed that there was a significant and inverse relation between these two variables in 0.002 level. Consequently, exhaust level would decrease by increment of physical readiness. But in $p<0.05$, there was no significant relation between physical readiness and psychic health. There was a significant relation between exhaust and psychic health in 0.001 level.
    2. Regarding to statistical analysis of averages for physical readiness and exhaust, the results showed that the group with higher physical readiness (83 scores against 17) had lower exhaust (53 scores against 62).
    3. Statistical analysis for prediction of variables showed that there was a significant and inverse relation between physical readiness and exhaust in 0.002 level, so exhaust can be estimated by physical readiness and some of its subsets like hearth strength and body fat. The regression equation obtained from this study is
       Predicted mark = -0.16 (physical readiness + 68)
- Etnier et al (1997) suggested that exercise can speed up blood flow and consequently increment of Nur-epinephrin and Dupamin that cause permanent changes in brain structures of animals. Some of these changes can affect oxygen supply of brain that consequently increases memory power and cognitive performance.
- Frew (1988) studied efficiency improvement and job consent through training programs for staff. The results showed that, after a training period and exercises, efficiency and job consent increased. Finally, he suggested that managers can increase job consent and hence staff efficiency by sport and recreational activities with a trivial cost.
- Kirkby et al (1999) showed that internal incentives to participate in sport activities would increase. They studied 280 Australian and 103 Chinese. Motivation factors for Australians were recreation and reward and for Chinese was a good body.
- Bowman et al (2001) suggested that the most important motive of athletes to direct toward sport are: learning new skills, improving previous skills, enjoyment of physical activities, eagerness of competition, and earning fame and rank.

- Coetzee et al (2002) showed that the most important motive of participant in sport activities were learning, improvement of skills, and progress.

Generally, regarding to the studies of Ministry of Science in the form of some projects, theses, Ministry of Education, and Ministry of Health, we conclude that motive and power variables transact with behavioral skills so that improvement of motive and power by physical situation will improve happiness and hence teaching method of teachers. In contrast, teaching consent will balance chronic stresses. However, there are many factors affecting this process that must be studied.

2. Material and Methods

The goal of this study is developmental, and study method is descriptive and geodesic, which study motive level among lecturers of Teacher Education Centers by field and library questionnaires. Spearman Correlation Method was used to determine the relation between study variables in subjects. The society of this study was all lecturers of Iran Teacher Education Centers. Samples were selected at first by state and certain centers non-randomly. Then the desired volume was selected randomly according to estimation criteria (30% of lecturers of each center).

- Tools of this study were:

1. A general questionnaire including: sex, age, record, education, program, and site.
2. Teacher poll about ways of motive increment, in the form of interview and questionnaire. Validity of this questionnaire was accepted by simultaneous retest by researcher.
3. A questionnaire for Mazlow Needs Hierarchy by Jones and Fifer (translated by Moghimi) was used, which examines type and motive level of subjects (in form of a triangle that is ordered from base as: 1. life need, 2. safety, 3. social belong, 4. personality, 5. self-prosperity). This questionnaire has been validated and used in country.
4. Maslach Exhaust Questionnaire with 25 questions that examines rate of motive in subjects by ignoring method. Questions 1-9 examine rate of emotional and corporal exhaust, questions 10-17 rate of performance decrement, and questions 18-25 rate of self-release. Lickert wealth spectrum was used to score questions. The score domain of this questionnaire was from 25 for least exhaust to 175 for most exhaust. This questionnaire has been validated and used in country.

- Measurement scale of this study is ordinal.

Descriptive statistic (frequency table and chart) and inferential statistics (Spearman correlation factor and Chi square) with SPSS and Excel were used to analyze data, and Power Point was used to display the results. The questionnaires were distributed by executer and et al in different Teacher Education Centers. Also related documents, review of different training periods, interview and poll were used.

3. Results

3.1. Sociodemographic data

According to our findings, Sex of subjects, age distribution, Job records, and their education level is shown in tables 1-3.

Table 1. Sex of subject

Sex	Quantity	Percent
Male	185	75.2%
Female	61	24.8%
Total	246	100%

Table 2. Age distribution of subjects

Age	Quantity	Percent
< 30	7	2.8%
31-40	79	32.2%
41-50	139	56.7%
> 51	20	8.2%
Total	245	100%

Table 3. Job records of subjects

Record	Quantity	Percent
< 10	7	2.4%
11-20	80	32.5%
> 21	157	63.8%
Total	243	100%

Table 4. Education of subjects

Education	Quantity	Percent
Bachelor	8	3.3%
Master	197	80.7%
PhD	39	16%
Total	246	100%

3.2. Motivation level of lecturers (Needs Hierarchy)

Observations of fig. 1 show the average of motive level (regarding to Mazlow's Need Hierarchy) was 2.7 among 246 teachers. This means teachers are mostly desired to "social belong." It must be mentioned that regarding to fig. 2, the average of motive level of females in social item is 3.32, and

regarding to fig. 3, that for males is 3.07. After satisfaction of life and safety needs, social security needs or belong are predominant. After that, persons generally like to become more than apparent belong.

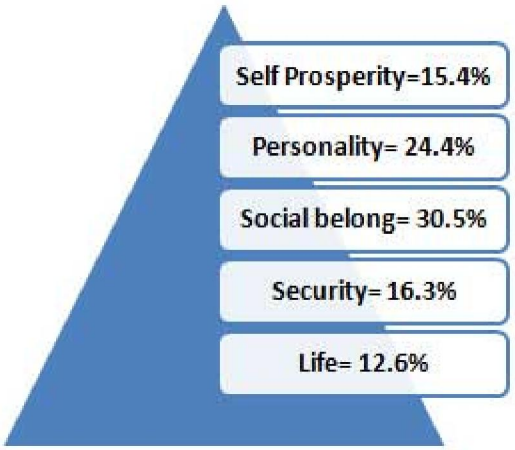

Figure 1. Testes needs hierarchy

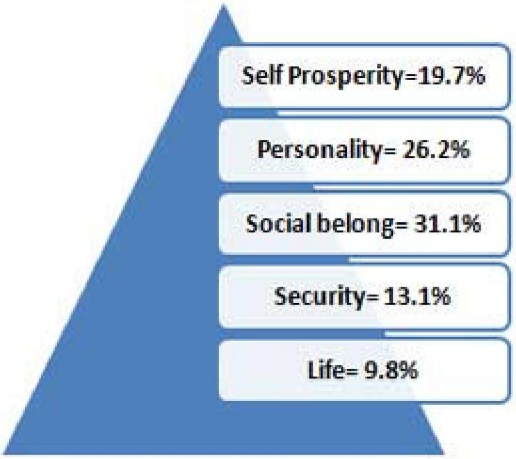

Figure 2. Female lecturer's needs

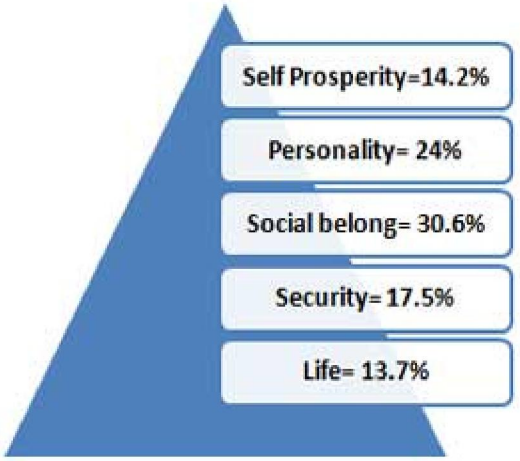

Figure 3. Male lecturer's needs

3.3. Motive rate of lecturers (exhaust)

Regarding to figs. 1 and 3, the average of motive rate (exhaust rate) is 55.47. This means that exhaust rate (emotional and corporal burnout, decrement of performance, self-release, etc) are good regarding to exhaust 175 (100%). In other words, motive rate of lecturers is acceptable. Also, the average of motive rate for females is 52.88 and for males is 56.33.

Table 5. Average of motive rate (exhaust rate)

	Average	Percent
Exhaust	55.47	31.69%
Female exhaust	52.88	30.21%
Male exhaust	56.33	32.18%

3.4. Correlation of variables

Correlation of variables is shown in table 6.

3.5. Comparison of motive level (need) and motive rate (exhaust)

The variables are compared in table 7. Observations of table 7 show that average of motive level (regarding to Mazlow's Needs Hierarchy) is 3.13 among 146 teachers. This means teachers are mostly desired to "social belong." This is similar for males and females.

3.6. Difference level of some variables

This is seen in table 8. Regarding to observations of table 8, difference of motive level (need) for male lecturers with sig=0.001 is more than females with sig=0.048, this shows diversity of needs of males is more than that of females.

Table 9 shows difference of motive rate (exhaust) between males and females. Regarding to observations of table 9, difference of motive rate (exhaust) for male lecturers with sig=0.001 is observable by themselves, but in females with sig=0.048, this has no significant level. This means males' exhaust is more than that of females.

Table 10 shows difference of motive level (need) with respect to teaching record. Regarding to observations of table 10, difference of motive level (need) for low-record lecturers with sig=0.043 is observable. However, in high-record lecturers this has no significant level. This means need diversity for low-record lecturers is more than that for high-record lecturers. Table 11 shows difference of exhaust level with respect to teaching record. Regarding to observations of table 11, difference of exhaust level for teaching record of lecturers is not significant. This means that job record has a low effect in exhaust.

Table 6. Correlation of variables

	Need	Sex	Age	Record	Educations	Exhaust
Need	–	–	–	–	–	–
Sex	–	–	–	–	–	–
Age	–	–	V	R=0.89 P=0.001	R=0.57 P=0.001	R=0.15 P=0.02
Record	–	–	R=0.89 P=0.001	–	R=0.39 P=0.001	–
Education	–	–	R=0.57 P=0.001	R=0.39 P=0.001	–	–
Exhaust	–	–	R=0.15 P=0.02	–	–	–

Observations of table 6 show that there is a significant relation between age and record (Spearman R=0.89) with p=0.001 or α=1%. This means lecturers' records will increase with their ages.

There is a significant relation between age and educations (Spearman R=0.57) with p=0.001 or α=1%. This means lecturers' educations will increase with their ages.

There is a significant relation between age and exhaust (Spearman R=0.15) with p=0.02 or α=5%. This means lecturers' exhaust will increase with their ages.

There is a significant relation between record and educations (Spearman R=0.39) with p=0.001 or α=1%. This means lecturers' educations will increase with their record.

Motive rate for females has similar situation, and it is also better than males.

Table 7. Comparison of motive level and motive rate

Number of subjects	Motive level	Females' motive rate	Males' motive rate	Motive rate	Females' motive rate	Males' motive rate
346	X=3.13	X=3.32	X=3.07	X=55.47	X=52.88	X=56.33
	S=1.24	S=1.32	S=1.24	S=13	S=11.07	S=13.50
	R=1-5	R=1-5	R=1-5	R=31-105	R=33-80	R=31-105
	R=1-5	R=1-5	R=1-5	R=25-175	R=25-175	R=25-175

Table 8. Difference of motive level (need) between males and females

	Females	Males
X^2 = Chi square	9.574	19.104
df = tolerance	4	4
sig = significance level	0.048	0.001

Table 9. Difference of motive rate (exhaust) between males and females

	Females	Males
X^2 = Chi square	21.033	93.940
df = tolerance	35	55
sig = significance level	0.970	0.001

Table 10. Difference of motive level (need) with respect to teaching record

	Low record	High record
X^2 = Chi square	9.8	11.7
df = tolerance	4	4
sig = significance level	0.043	0.20

Table 11. Difference of exhaust level with respect to teaching record

	Low record	High record
X^2 = Chi square	5.267	6.733
df = tolerance	22	18
sig = significance level	1	0.992

3.7. Views of lecturers for recognition and prioritize ways of motive increment

A questionnaire made by the researcher was used to examine views of lecturers for recognition and prioritize ways of motive increment. This questionnaire had a multi-option close question and an open question. The results are shown in table 12.

Most lecturers voted to "salary increment" to increase motive of lecturers, and "meritocracy", "appending Teacher Education to Ministry of Sciences" followed that. It seems that "correct planning" is in suitable level.

4. Discussions

4.1. Motive level examination

Regarding to fig. 1, most frequency belongs to social belong and least frequency belongs to life level. This is similar for males and females. After satisfaction of life and safety needs, there are supply of social needs or belong. In this structure, since human is a social creature, he needs belong and desires to be accepted by various groups. When social needs predominate, human tries to find significant relations with others. After commencement of belong needs, persons want to be a member of a group officially and more than an apparent belong. Hematinejad et al in a similar

study in 2001 propounded self-prosperity and social needs as first and second priorities.

Table 12. Poll to recognize and prioritize ways of motive increment

Priority	View	F	F%
1	Increment of salary	143	15.5%
2	Meritocracy	102	11%
3	Appending Teacher Education Center to Ministry of Science	100	10.8%
4	Efficient management	86	9.3%
5	Jog security	83	9%
6	Respect to lecturers	81	8.7%
7	Suitable training space	60	6.4%
8	Efficiency and efficacy	59	6.4%
9	Participation in decision-making	54	5.8%
10	Peripheral facilities	36	3.9%
11	Delegation	27	2.9%
12	Diversity of teaching	22	2.3%
13	Respect to precedent	18	1.9%
14	Job independence	18	1.9%
15	Environment health	14	1.5%
16	Improvement of evaluation system	10	1%
17	Correct planning	8	0.8%
		N=921	N=100%

Sanei et al in 2001 have propounded social belong and first priority. Regarding to average of motive level index (needs hierarchy), 30.5% of lecturers showed their desire to "social belong". As human essentially desires to make relation with others, lecturers also need mutual understanding of organization and them. In contrast, regarding to job nature of teaching, communications and social belong must be predominant. Probably weakness of meritocracy, inefficient management, unsuitable encounter without respect, weakness of efficiency and efficacy, or lower share of lecturers in decision-making may encouraged this need.

4.2. Examination of exhaust rate

Table 5 shows exhaust level of 31.69%. This means that exhaust rate is low and motive rate is acceptable. Regarding to high age average and record of subjects and direct relation of age and record with exhaust rate, their motive rate should be preserved. Obviously, directors must effort to keep this situation. In a similar study, Sadeghi Borujerdi in 2003 showed that the above statistical sample had a significant exhaust which its main cause was job stressful factors.

4.3. Examination of teaching record

Table 3 shows average of records of subjects (63.8%) above 21 years old. This shows that there are high record lecturers in the centers, and this situation has opportunities and threats. Opportunities are mature, high experience, and low exhaust level. Threats are lack of support and early retirement.

In a similar study, Sane'ei et al in 2001 showed average of 167, which is a power point to plan quality improvement. Also Nurbakhsh in 1997 observed a significant difference between teaching record and hygiene-motivation factors.

4.4 Comparison of motive level, teaching record and exhaust rate

As showed in table 7, there is no difference between two female and male lecturers for motive level and motive rate variables. In a similar study, Nurbakhsh in 1997 observed a significant difference between teaching record and hygiene-motivation factors. Also, Karimi Kalimani in 1998 claimed that F test with α=5% shows that except job record, there is no difference between motives and personal features. Regarding to findings and study records for above variables, it is probable that male and female view, needs, and desires be similar. Consequently, sex is the next priority in macro planning for males and females, and the first priority is removal of needs for both. In table 8, difference of motive level (need type) of males and females is significant. This means that diversity of needs of females is more than that of males. Therefore, it is recommended that planners notice needs of males and females and Mazlow's Needs in short-time plans. In table 9, difference of exhaust level of males is significant. Therefore, it is recommended that planners notice diversity of different aspects of job, social, economical and cultural differences and problems for males, which this is because of responsibility of males in their individual and family lives. Table 10 shows difference of needs of low record lecturers. This shows extent of needs of young lecturers in their first years of service, which directors must notice to this aspect, because their needs is noticeable because of low salary and formation of family. Table 11 shows difference of exhaust level of low record lecturers with high record lecturers is not significant. Consequently, difference of record had no significant role in exhaust level of subjects.

4.5. Correlation of study variables

Table 6 shows that there is not a significant relation between motive level of teaching record and exhaust rate. Also, there is a significant correlation between age and record with R=0.89, P=0.001 and α=1%. Also, there is a significant correlation between age and education with R=0.57, P=0.001 and α=1%.

There is a significant correlation between age and exhaust with R=0.15, P=0.02 and α=5%. Finally, there is a significant correlation between education and record with R=0.39, P=0.001 and α=1%. The results show that increment of age has a strong relation with record, education, and exhaust, because increment of record provides opportunity for these situations. Also, there is a strong relation between increment of record and education level, but threats like increment of exhaust level and expectations will also be created. In a similar study of Kurosh Mafakheri in 2002, knows values as a determinant factor of motivation factors. He knows self-preservation as the most essential factor, and celestial love and the highest motivation. Also, Sadeghi Borujerdi in 2003 suggests exhaust and psychic-corporal health as an important factor on both cases.

Table 13. Brief of findings and proposed styles to improve motive in subjects

Brief of findings	Proposals
– 75.2% of subjects are male. – 56.7% of subjects are above 41. – 63.8% of subjects have more than 21 years record. – 80.7% of subjects have master degree. – Exhaust level of subjects is 31.69%. – Exhaust level of female lecturers is 30.21%. – Exhaust level of male lecturers is 32.18%. – Social belong (30.5%) and personality (24.4%) are main needs of subjects. – Social belong (31.1%) and personality (26.2%) are the most important needs in females. – Social belong (30.6%) and personality (24%) are the most important needs in males. – There is no difference for motive level and rate variables in males and females. – Difference of motive level between males and females is significant in their groups. – Difference of needs between low record and high record lecturers is significant. – There is significant correlation between age and record, age and education, age and exhaust, and record and education. – The most important views: 1. Increment of salary (15.5%). 2. Meritocracy (11%) 3. Appending Teacher Education to Ministry of Sciences (10.8%). 4. Efficient management (9.3%). 5. Job security (9%). 6. Esteem to lecturers (8.7%). 7. Suitable training space (6.4%). 8. Efficiency and efficacy (6.4%). 9. Participation in decision-making (5.8%). 10. Peripheral facilities (3.9%). 11. Delegation (2.9%). 12. Diversity of teaching (2.3%). 13. Considering job record (1.9%). 14. Job independence (1.9%). 15. Environment hygiene (1.5%). 16. Improvement of evaluation system (1%). 17. Correct educational planning (0.8%). 18. Improvement of motives of lecturers by Integral System of Training Education specially by article 8 of Training Education System considering "service motive" for a good teacher.	– Do not accept early retirement request of lecturers. – Participation management is the best option to make relation with lecturers (regarding to priority of social belong need). – Complete execution of uniform system of salary payment. – Formation of a database of lecturers. – More attention to research and removal of obstacles and challenges of study in Teacher Education Centers. – Rewarding active lecturers in special days like Teacher Week and Research Week. – Regular examination of mental and corporal health of lecturers. – Provision of sport and recreational plans for leisure time of lecturers. – Evaluation of lecturers situation in each educational term. – Forming SWAT Committee in each center to analyze weakness and strength points, opportunities and threats affecting on lecturers: Strength Weakness Opportunity Threat – Determination of level, rate, and direction of needs of lecturers and deciding to remove them. – Removal of culture of "one prescription for all" and replacing culture of "one prescription for one". – More notice to low record and young lecturers. – Notice to problems of male lecturers if they are head of a family. – Notice to differences of needs of males and females. – Regarding to this that most lecturers have master degree and low exhaust, this is the best opportunity for directors to use them in managerial activities.

4.6. Views of lecturers for recognition and prioritize ways of motive increment

Table 12 shows there is 17 priorities for motive improvement of lecturers, which the most important ones are salary increment (15.5%), meritocracy (11%), and appending Teacher Education Centers to Ministry of Sciences. The first priority, namely salary increment, may be because of uncompleted execution of uniform system of salary payment in comparison with other ministries including Ministry of Sciences. Since student of Teacher Education Centers enter to university

through global Konkur examination of Ministry of Sciences, and naturally syllables of teaching for lecturers are similar to that of university professors, therefore subjects expect there is no discrimination. On the other hand, the peripheral bonus for professor of Ministry of Sciences does not exist for lecturers of Centers (including membership in Scientific Board, science environment of universities, research-orientation, improvement of educational level, welfare affairs like residence, high speed internet, publications, diminish of work hours, welfare of family, university independence, short-term, mid-term, and long-term study opportunities, facilities to participate in exhibitions, scientific camps, authoring lesson books, strong structure, etc). Need to meritocracy also may be cause of inefficient selection of directors. In a similar study, Sane'ei in 2004 suggests first to third priorities.

4.7. Styles of motive improvement in lecturers of Teacher Education Centers by main variables, poll, theoretical record and study record

Result of poll of lecturers shows that they have answered the questionnaires regarding to their needs, desires and inadequacies. It means each proposal comes from a limited group, and it is not a major problem of Teacher Education. Consequently, this is duty of directors and planners of Teacher Education to contrive strategies through gathering queries.

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A review of the validity and reliability of assessment instruments for the final examination for the midwifery students of nursing and midwifery faculty of Mashhad

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Abstract: According to WHO, the annual mortality rate for mothers is 515000 cases in the world. Most of these deaths can, however, be prevented when skills and knowledge are enhanced in the delivery ward. The poor performance of the delivery supervisors in Iran can be held accountable for most of the mother deaths. The lack of an objective tool reduces the possibility of a systematic and valid evaluation of the clinical examinations particularly at postgraduate programs. Therefore, the present research was conducted to investigate the validity and reliability of assessment instruments for the final comprehensive examination for the midwifery students. In this study, based on the checklist available at the faculty, the students' skills were studied in four test fields (delivery room, clinical gynecologic unit, parental care unit, and mother and children health). Thirty one students (22 from term eight and 9 students from term six) were selected in five consecutive days by two examiners (one of the examiners had no knowledge about the educational level and scientific knowledge of the students). For the face validity and content validity, the experts in the field were consulted. In addition, the criterion validity (the correlation between the score means in the clinical and theoretical courses and the students' total average from the final comprehensive examination) and the construct validity were separately calculated. To calculate the internal consistency, the Chronbach's alpha coefficient and the equivalence reliability (the correlation between the scores was separately reported by the two examiners) were used. The correlation coefficient in the final comprehensive examination of the midwifery program with an average score of 0.22 and 0.52 (p=0.01) for theoretical and clinical courses, respectively proved to be the desirable criterion validity in the assessment forms. Besides, the face validity and the content validity with a high reliability index (0.6-1) were confirmed. The occurrence of high to middle Alpha coefficient reliability (0.51-0.83) in every field of the examination showed an internal consistency in the available checklist. Following the definition of the criteria for the values of the scores, the lowest, and the highest reliabilities were determined to be 0.82 and 73.5%, respectively. This study concluded that the assessment instruments of the final comprehensive examination at the bachelor degree for the midwifery students at the nursing and midwifery faculty in Mashhad, Iran, are recommended as the valid and reliable forms for the assessment of the students at their educational career.

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1. Introduction

A major part of the educational process is to assess the academic progress and evaluate the amount of the students' learning. As the definition runs, the assessment of the educational progress is to evaluate the performance of the learners and compare the results with the predetermined educational objectives in order to decide whether or not these educational activities lead to desirable results; whether the intended goals have been achieved and to what extent the goals have been achieved (1). Chronbach has collected the assessment and defined the application of the data in order to decide on the educational program (2). So it is necessary to make sure about the appropriateness and compatibility of the assessment methods with the goals specific to learning. Unfortunately, there are some shortcomings in the way the clinical regular examinations are held which may include the high number of participants in each exam, the short and limited time allocated for the evaluation and also the non-objectiveness of the evaluation (3).In a research study conducted in 2001 on the dissatisfaction of the medical students with the way the evaluation is made, Bahreini reported a 46.6% non-correspondence in the final score with the skills acquired. About one third of the trainees

emphasized the necessity of reviewing and changes in the assessment (4). This dissatisfaction was also sometimes expressed by the clinical trainers. For example, in an assessment of the nursing clinical skills in Indonesia (2007), about 20% of the trainers expressed dissatisfaction with the method of evaluation and about 21% of the trainers felt sorry for the failure of the students in the final examination due to their judgments. Even 52% of the trainers reported that they held themselves accountable for the failure of the students in the final examination (5). However, the importance of the desirable evaluation of the midwifery students becomes known at the graduation when it is necessary to pay attention to consequences of the lack of essential skills in controlling the women especially the pregnant ones. According to the World Health Organization (WHO), the annual occurrence of 515000 cases of deaths in mothers in the world indicates that it is required to provide more training and further delivery skills (5). According to the statistics of 2002, Iran also stresses this point. Although 80% of the delivery is conducted by the university-educated people, the case study on the mortality of the mothers shows that there is malfunction in the delivery ward (6).Attention to these facts can enhance the sensitivity and attempts of the clinical midwifery trainers in reviewing the required midwifery skills. Granland & lyn (1990) classifies learning skills as the career skills that cannot be assessed by the common examinations and believes that practical tests are required in this regard (1) For the evaluation of the clinical qualifications, there have been various suggestions such as portfolio, objective structure clinical skills (OSCE), direct observation, written examination etc. (7).

One of the practical tests is the portfolio. To show his capabilities and progress and for the sake of evaluation, the learner provides the instructor with some samples of the work he has done during a certain period of time. DEMBO (1994) says that one of the obstacles to these modern methods of evaluation is the determination of the validity, reliability and the style of evaluation in different steps. Performance in the simulation situations can be one of the practical tests. In this method, the learner is required to perform an activity in a simulated and artificial situation as he does in the actual one (1). Today, for most of the clinical skills, the OSCE has been designed. The advantages of this method include the consistency of the examination situation, the standard patient, and lack of stress. However, before these tests are conducted, it is necessary to have a suitable and reliable checklist.

Another type of practical test is the direct observation which facilitates the assessment of the clinical skills of the medical sciences group. However, in the study of the specialized clinical techniques via the direct observation method, there is a doubt, in fact, an objection which is the result of the non-centralized nature of this instrument and the effects of other variables such as the inclination of the examiners, a change in the behavior of the examinees and so on. Therefore, for the enhancement of accuracy in the process of observation, the use of the DOPS system is recommended. In this method, in addition to observing the learner at work, the academic staff has to pay attention to checklist items which are under evaluation and are pre-designed. At the end of the work, the evaluators must flag the appropriate items in the checklist to specify the results of their observations (8). Thus, the checklist method is an organized style for reporting the observer's or observers' judgments. COOPER (1994) stressed the feature of "being important" as one of the characteristics which should be evaluated in the checklist method. In the checklist method what is recorded is just the presence or absence of the feature or its quantity. The attributes of a good checklist include the brevity and clarity of and the emphasis on the observable behavior and recording of the important behaviors. Like the checklist method, the scaling method is used for the facilitation of the observer's evaluation of the performance or style of the application. Other effective factors in improving the scaling method include checklist, being important, observability, and clarity of the points on the scales and unaccountability of the evaluators in case of doubts. In addition to the method for the evaluation of the clinical qualifications which is mainly based on the conditions and practicality of its application, it is necessary to pay attention to the criteria of a fair examination such as validity, reliability and objectivity (9) Based on the definition, a test is claimed to be valid if it can measure what it is intended to measure. However, by reliability it is meant that the instrument can produce identical results in identical conditions at different times. Most sources emphasize that in all kinds of research, the reliability instrument should be reviewed whether it is a survey, a mechanical instrument or a human observer (10). In the study of the skill techniques, the Royal College of Women and Midwifery (2006) focuses on the validity and reliability of the instrument, and recommends that in any instrument the construct validity, content validity and face validity be concurrently tested and the evaluators' agreement be sought (11). With an instrument having validity and reliability, it will be possible to be sure about the proper evaluation which will finally be achieved by an increase in the satisfaction of the students and the evaluators.

Now, in the nursing and midwifery faculty in Mashhad, Iran, for the clinical evaluation of the midwifery students, the observational instruments along with the checklist are used in four fields of delivery room, clinical gynecologic unit, parental care unit, and mother and children health. In a study on the percentage of satisfaction of the midwifery students with the final examination in 2008, Farokhi et al reported that the degree of satisfaction was 80, 71.9, 75.75, and 67.4 in four fields of delivery room, clinical gynecologic unit, parental care unit, and mother and children health respectively (12). Hence, as the studies show, the researchers consider the validity and reliability measures and the way the evaluation is made as the first educational priority of this faculty for the enhancement of the satisfaction of the students and the instructors This can guarantee the objectivity and fairness of the examination, because lack of objective goals and an objective instrument can not only reduce the possibility of systematic and valid assessment of the students at the time of graduation, but it can also make it impossible to make arrangements for interventions to boost the performance of the students after graduation. Therefore, this research has been grounded on the fact that there is limited amount of research in this field and the results of this study can lead to the improvement of the quality of clinical education with special emphasis of the educational process on the validity and reliability of the instrument of evaluation.

2. Material and Methods

In this research, for the study of the validity and reliability of the evaluation forms of the final comprehensive examination of the midwifery students, one week before the official final comprehensive midwifery examination in the faculty, with the help of these instruments, an exam was held in four fields of delivery room, clinical gynecologic unit, parental care unit, and mother and children health. In each exam, there were two examiners one of whom were from outside the faculty and were unaware of the study level and knowledge of the students who were going to be assessed. At the beginning, the examiners were requested not to talk with each other about the educational term and their previous acquaintance with the study students before the forms were completed. In inviting the external examiners (experts working in health centers or Azad University), the researchers tried to choose those whose field of interest and expertise corresponded with the type of the examinations. When they expressed readiness to cooperate in this filed, first they had to have a two-hour-long session to get acquainted with the content of the forms of the final comprehensive examinations and learn how to fill in the forms and how the forms were graded by those in charge of midwifery at the presence of the researcher. After the examiners became fully familiar with how to fill in the forms, these instruments were administered for five apprenticeship students in four different fields. Then the respective examiners with the help of the instructor of that field completed the forms and determined the correlation coefficient. After the determination of the correlation coefficient ($r>0.7$), at the time of a similar final examination, the examiner became qualified to administer the exam with the help of one of the instructors of the group (the two-member groups of evaluation were specified with the nearest correlation coefficient would determine the study research units of all of the applying students of term eight. The participating students of term 8 had to have passed all the credit units of the undergraduate program. They were supposed to be introduced for the final comprehensive examination by the administrative department. The students of term 6 also had to have passed all the credit units except for the field course. In this research, for the evaluation of validity and reliability, different types of validity such as content validity, face validity, criterion validity, construct validity and equivalence reliability and internal homogeneity were taken into consideration. To statistically analyze the obtained scores, the study units were converted to percentage by means of the checklist and the scaling methods. The variance analysis test and t-test were used to compare the means and the correlation coefficient and Chronbach's alpha. After the educational goals were specified on the basis of the objectives of exam courses, the evaluation instruments in terms of content validity for the final examination of the midwifery students were placed against each question as Necessary, Useful and Not necessary. The questionnaire was given to five experts in the field and they were requested to choose the appropriate item for each question. According to the formula (LAWSHE formula), the content validity Index (CVR) was determined. If the index turned out to be positive, it signified that there was some validity in half of the questions. For the face validity, the experts in the field were consulted and the academic staff would determine the content validity. What would be used for the criterion validity included the validity coefficient or in other words, the correlation coefficient between the means of the theoretical and clinical scores of research courses and the scores obtained in the comprehensive examination as well as the correlation of the total score with the means of the theoretical and practical courses. A validity index of 7% signified the presence of criterion validity.

In order to verify the construct validity, the known-groups-technique was used. Therefore, the mean of the obtained scores of the study students would be evaluated. If the highest score belonged to the students who were at the highest level of educational program (students of term eight), there was a statistically significant difference and so the construct validity was verified because it showed that this instrument was able to differentiate between the individuals with different educational levels.

In order to determine the equivalence reliability, the exam was administered by two equivalence evaluators and it happened at the same time (with the direct observation of the performance). Then, the correlation coefficient was determined. For the internal homogeneity, the Chronbach's alpha was used. Since the final examination consisted of 40 sub-tests, the score variance of each part and the variance of the total score were calculated.

3. Results

In this study, 70.9% (22 individuals) of the participants were in term eight and started their program at nursing and midwifery faculty in 2006 and 29% (9 individuals) of the students were in term 6. The average age of the student participants was 22 (i.e. 20-25). 53.3% of them were married and 46.7% lived on campus. 80% of the students of term eight were fully satisfied with the style of final examination. In response to the questions related to the final comprehensive examination, 73.3% said that they were familiar with at least one of the examiners and 93.3% were familiar with the clinical atmosphere of the examination. In 80%, the examinations in four fields were held in the morning and the average time length of evaluation was 3.1 hours. The amount of perceived stress before, during, and after each field of the examination was calculated out of 10 which turned out to be 6.6., 4 and 2.8 respectively in the prenatal care unit. Table (1) shows the scores for the perceived stress in other exams.

Table1. A comparison of the mean of the stress scores in the final comprehensive examination according to time and field of examination

Field of exam / Stress score	Health	Women	Delivery room	Prenatal	Result of tests of analysis and variance P	dF	F
Before exam	7.2±2.6	6.3±2.7	6.7±2.7	6.6±2.2	.42 0	3	.950
During exam	5.7±2.6	3.9±1.9	4.5±3/.	4±2.7	.24 0	3	1.41
After exam	4±3.2	2.8±2.3	2.4±2.1	2.8±2.7	.43 0	3	.920
Result of tests of analysis and variance	F=2.24	F=1.67	F=1.6	F=1.6			
	Df=2	Df=2	Df=2	Df=2			
	P=0.11	P=0.19	P=0.20	P=0.001			

Table 2. The difference in the mean of scores in the test fields of the final comprehensive examination among students of terms 6 and 8.

	Students of term 6	Students of term 8		
Test fields	Mean±SD	Mean±SD	Difference in percentage	Result of Mann-whitny test
Prenatal	14.4±0.9	15±1.9	15±1.9	0.26
Delivery room	9±2.5	14.7±1.2	0.63	0.00
Women	12.8±2	13.7±1.8	0.06	0.26
Mother and child health	9.5±1.2	14.6±2	0.53	0.09
Final total score	45.7	58	0.26	0.01

Eighty percent of the examiners (15 individuals) from the academic staff who participated in running the examination were over 40 or so. Their average teaching experience was 12.3 years (1-23). The average age of the evaluators was 25 years with an average teaching experience of 2.5 years (1-3). In all cases, the examiners and evaluators had a master degree. There was a statistically significant difference between the age and the teaching experience of the examiners and the evaluators (p=0.001). 73.3 % of the examiners emphasized the necessity of the final comprehensive examination in the field of midwifery. 33.3% of these examiners believed that if the students failed in this exam but proved their capacity during apprenticeship, it is essential to investigate the condition of running the final examination and finding out the reasons for the students' failures in the examinations.

The face validity of all of the assessment forms in different fields (delivery room, clinical gynecologic unit, parental care unit, and mother and children health) was confirmed by five midwifery specialists. The content validity of the assessment forms in the final comprehensive examination of the midwifery was also calculated by means of the LAWSHE formula in all cases except the following: In the prenatal assessment form in the questions related to establishing an appropriate physical atmosphere for individual features, the validity coefficient was 0.6 and in the assessment form of the students at the delivery room, the individual features and answers to the scientific questions had a validity coefficient of 0.6. In the assessment form for mother and children health related to continuation of giving milk and investigation into the nutrition of the breastfeeding mother and the control of the refrigerator and answer to the scientific questions, the validity coefficient was 0.6.and the

Table 3. Reliability of the components of the final comprehensive examination of midwifery based on internal consistency index

Test fields	**Alpha index**
Prenatal	0.27
Delivery room	0.69
Women	0.45
Mother and child health	0.16

Table 4 Reliability of the components of the final comprehensive midwifery examination according to the consistency coefficient index

Test fields	Alpha index
Prenatal and Delivery room	0.79
Women	0.68
Mother and child health	0.82

Only one item (number 1, related to the individual features) in the assessment form of the women unit was deleted due to the lowness of the validity coefficient (0.2). Following the comment by the experts, the assessment forms of the final comprehensive examination were modified.

4. Discussions

The results of the study show that the highest stress perceived by the students before, during and even after the exam lies in the mother and child health unit. The students experienced the least amount of stress during the exam in the women field and the least amount of stress after the exam in the delivery room unit (Table 1). Therefore, although the amount of satisfaction of the students of the nursing and midwifery faculty with the way the final comprehensive midwifery examinations were held in 2010 was relatively good (80%), with the improvement of the conditions and investigation into the differences between the exam units, there was a chance for promotion in the degree of student satisfaction. Since the 2008 attempts to enhance satisfaction, there has been an increase from 73.3% to 80% in the total satisfaction since 2010. Due to the importance of such an examination deemed by the 73.3 % of the midwifery instructors, it is necessary to make more investigations into this field. In a study on the way the nursing students are clinically evaluated, VENDA (2007) reported a figure of 6.1% of student dissatisfaction among 271 students in four educational centers in Jakarta. Although the evaluators reported the use of checklist in 75% of cases and the other methods such as video recording of the clinical reports and, on average, 3.3 hours (1-3) of evaluation per student were calculated, the students believed that the assessment score and the level of anxiety and stress depended on who evaluated them and how. This researcher finally says that for a desirable evaluation and enhanced satisfaction, it is necessary to take four factors into account: instructors, clinical atmosphere, evaluation methods and finally the students themselves. Differences in students demand differences in evaluation methods. Some of the solutions recommended by this researcher include the oneness of the evaluators and the examiners, the clarification of the educational goals, the increase in the number of evaluators and the duration of the evaluation, and encounter with similar cases during the clinical apprenticeship (5). Regarding the emphasis of most of the midwifery instructors (73.3%) on the necessity of running this test, it can be said that based on the four aforementioned factors, it is possible to achieve a higher level of satisfaction especially in the reduction of anxiety and stress in the students of the faculty.

The difference in the performance of the students of terms 8 and 6 in Tables (3-7) has shown that the results state that in the prenatal unit, the students of higher terms had an increase in score mean in examining the patients, extracting history, assessing and performing the special care. Although it was reported that this difference was only statistically significant in examining the patients, ($p=0.03$), the total average obtained by the students of term 6 was lower compared with that of the students of term 8 (14.4 ± 0.9 against 15 ± 1.9). Furthermore, unlike the expectations, the students of term 6 had a better performance, so with some training of this skill in the women unit in the lower terms, the students should have further awareness of the importance of giving training to the patients as a

way to prevent the dangerous side effects during pregnancy.

Other results of this research indicated a significant difference between the skill and performance of the students of years three and four in midwifery in all parts of the final comprehensive examination during delivery room unit (Table 4) shows the significant difference (p=0.00) in the average total score obtained by these two groups (9±2.5 against 14.7 ±1.2)

A comparison between the performances of the students in the field of women showed that there was a significant difference between the students of years three and four at the undergraduate program in midwifery in terms of examining the patients and the internal genital tract. There was a difference between the average score obtained by two groups of students (12. ±2 against 13.7±1.8). Although this unit was not statistically significant, the students of term 6 got a higher score in uterine examination and smear test and diagnosis.

The result of this research in the performance difference of the high and low term students showed that in the mother and child unit, the skills of the students in the fourth year of undergraduate program in midwifery improves in three main fields: children's nutrition, vaccination technique and family planning. This difference was statistically significant (Table 6). Besides, the mean of the scores obtained in other items of assessment including investigating the children's common problems, special care, completion of files and records showed that the students of term 8 got a higher score. Although this difference was not statistically significant, there was a statistically significant difference between the total scores of students of term 6 and 8 (p=0.02).

The Royal Women and Midwifery College (2002) mandates the construct validity for each instrument for the assessment of skills. An instrument is claimed to have construct validity when it can differentiate the population with the intended characteristics from the population lacking those features. Accordingly, this college considers a test as having construct validity when it can distinguish the trained individuals from those who are not and it can even grade and compare the levels of the learners' education (higher or lower) (11). In an investigation into the skills of 31 surgeon assistants of years three and four in Toronto, FRIDLEECH (2002), using OSCE with checklist and Likert Scale, confirmed the construct validity in the checklist and scaling forms because there was a statistically significant difference (p=0.001) and the mean difference was 0.25% (79% against 89%) between the senior and junior students. The comparison between the results obtained in this study showed that there was a significant difference by 26% in the total score mean in the final comprehensive examination of the students of term 8 (Table 7). Therefore, based on the definition given by the Royal Women and Midwifery College, it is confirmed that in this examination there is construct validity in the assessment forms of the midwifery students (13).

The Chronbach's alpha is used to calculate the internal consistency of the measuring instruments such as the questionnaire or the tests which measure different properties. For the calculation of the Alpha coefficient, it is necessary to calculate the variance of the scores of each subgroup of the questions and the total variance. The zero value shows lack of reliability and +1 indicates total reliability. So in this research, the Chronbach's Alpha internal consistency coefficient is used for the investigation of different fields of the test (prenatal, delivery room, women and mother and child health)(Table 9-12). These coefficients were determined as 0.77 for the prenatal unit, 0.83 for the delivery room, 0.50 for the women unit and 0.63 for the mother and child health. The results of this research also showed the average-to-good structure and consistency between the subtests of the final comprehensive midwifery examination. Of the factors which had effective roles in boosting reliability of the test were the length of the examination and identical questions in terms of content and average level of difficulty (17). Therefore, for the re-evaluation, the researchers compared the total scores of pregnancy and delivery (delivery room and prenatal) units with other test units in terms of internal consistency reliability. The coefficients indicated an increase in the total internal consistency, which is recommended by the researcher for the improvement of the internal consistency in the final midwifery examination.

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Evaluation of Euphorbia Aphylla, Ziziphus Spina-Christi and Enterolobium Contortisiliquum as Molluscicidal Agents

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Abstract: The present work was carried out to evaluate the molluscicidal activities of ethanoic extract of three medicinal plant species namely *Euphorbia aphylla*, *Ziziphus spina chriti*, and *Enterolobium contortisiliquum* against *Biomophalaria alexandrina* and *Lymnaea cailliaudi* (*nalatensis*) snails the intermediate hosts of schistosomiasis and fascioliasis respectively.the experiments were conducted in accordance with WHO guidelines. Probit analysis was used to determine the $LC_{50}$and LC_{90} after 24 hours exposure. The highest molluscicidal potency was recorded for *E. aphylla.* It exhibited significant molluscicidal activity on both snails' species. The LC_{50} and LC_{90} of this extract against *Lymnaea cailliaudi* were 0.66 and 0.88 ppm respectively and 87.6 and 142.5ppm against *B. alexandrina* followed by *Ziziphus spina- chriti* which showed molluscicidal activity against *L. cailliaudi* with LC_{50} 311ppm and LC_{90} 500 ppm and caused no mortality of *B. alexandrina* up to1000 ppm. The least active was *Enterolobium contortisiliquum* which gave negative results against both snail species up to1000 ppm. Further purification of active compounds present in *Euphorbia aphylla* and *Ziziphus spina- chriti* may eventually be of great value for the control of snails' intermediate hosts of fascioliasis and schistosomiasis.

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Key words: Plant molluscicides- *Biomphalaria – Lymnaea-* fascioliasis - schistosomiasis -Egypt.

1. Introduction

Schistosomiasis remains as one of the world's most prevalent diseases (King and Dangerfield-Cha, 2008). It is estimated to infect 207 million people worldwide. Approximately tenth of the world population are living with the risk of infection (WHO, 2010). In Egypt, the disease is not only a prime health problem, but it is also an economic one, as it affects million of farmers at the early age diminishing their productivity and exerting a serious socioeconomic problem(El-Baz *et al.*,2003). *Biomophalaria alexandrina* as specific intermediate host of *Schistosoma mansoni* is prevalent in both Upper and Lower Egypt (WHO, 2002).

Fascioliasis is a worldwide zoonotic disease caused by another trematode parasite of the genus *Fasciola* that infects over 17 million people causing significant morbidity and mortality (Mas-Coma *et al.*, 2005; WHO, 2006). In Egypt, fascioliasis becomes hyperendemic and problematic where animal reservoir and snail vector are available (Rashed *et al.*, 2008). Nearly 24 million Egyptians are at risk and about 800 000 suffering from fasciolosis (WHO, 1995, Haseeb *et al.*, 2002).Human infection causes serious hepatic pathological consequences (Soliman, 2008). In addition, fascioliasis is responsible for economic losses estimated at around one billion Egyptian pounds per year (Haseeb *et al.*, 2002). In general, El Shazly *et al.* (2002) found concomitant infection between fascioliasis and Schistosomiasis *mansoni*.

Treatment of *Schistosoma* and *Fasciola* infections remains highly problematic. In schistosomiasis, praziquantel is faced with failure to prevent reinfection as a result of development of drug resistance *Schistosoma* strain and serious side effects. Treatment of *Fasciola* requires high or multiple doses of drugs with frequent side effects (Ismail *et al.*, 1999 and Abdul-Samie *et al.*, 2010). Therefore snail control is considered not only complementary but essential in *Schistsoma* and *Fasciola* control. It is regarded as a rapid and efficient method for reducing or eliminating transmission and is among the methods of choice to bring these diseases under an adequate control through the breakage of the life cycle of the parasite (Mello-Silva *et al.*, 2006; Jigyasu and Sing, 2010).

Today, molluscicide is regarded as an important aggressive strategy in the control of the snail hosts of these diseases (Giovanelli *et al.*, 2001, Mello-Silva *et al.*, 2006). Unlike the use of synthetic drugs, the uses of molluscicides prevent reinfection of people after treatment (WHO, 1993). Copper sulfate and niclosamide were used in Egypt within a program developed by Bayer AG, however, due to their hazardous enviromental effects ,their toxicities to non-target organisms and even man, they were stopped (WHO, 2002 ; Abdelrazek *et al.*, 2007).Therefore the search for alternative molluscicides is still ongoing. During recent years

much attention has drawn for the use of molluscicides of plant origin. The use of plants with molluscicidal properties appears to be a simple, inexpensive and safe alternative (Singh and Singh, 2010; Al-Daihan, 2010). Also, there is a continuous need to search for new plant species with ideal molluscicidal properties (Tantawy *et al*., 2004; Bakry and Hamdi, 2007). In Egypt, several local plant species screened and proved to have molluscicidal properties against different snail species e.g. *Ambrosia maritime* (Abou Basha *et a1*., 1994), *Solanum* species (Tantawy *et al*., 2000), *Commiphora molmol* (Abd-Allah *et al.* 2009), *Guayacum officinalis, Calatropis procera* and *Euphorbia splendens* (Bakry, 2009).

Euphorbia is the largest genus of flowering plants in the Egyptian flora (El-Karemy, 2008). Over the past twenty years, they have received considerable phytochemical and biological attention (Wu *et al.*, 2009). According to Mwine (2011) a good number of *Euphorbia* species are actually potent as medicinal plants and their extracts have been isolated and patented as modern drugs. They have a variety of uses, such as for the treatment of intestinal parasites (Appendino and Szallasi, 1997 and Shi *et al*., 2008). They also possess antiamoebic (Tona *et al*., 2000); anti-plasmodial (Tona *et al*., 2004) and anti-leishmanial activity (Ahmed *et al*., 2006).Earlier studies indicated that the euphorbiales have molluscicidal activity (Tantawy *et al*., 2004; Sermsart *et al*., 2005; Bakry 2009; Singh and Singh, 2010). Alkaloids and saponin are reported among active compounds of several *Euphorbia* species (Siddiqui *et al*., 2009).

Ziziphus spina-christi is one of the plants most commonly used in Egyptian folk for treatment of different diseases and is traditionally used in Arab countries as a medicinal plant (Rigal *et al*., 2006 and Nawash and Al-Horani, 2011). In field of parasitology, the ethanolic extract of *Ziziphus spina-christi* root showed anti-schistosomal activity(Aly *et al*.,2006,El - Rigal *et al*.,2006).Anti-*leishmania* activities of ethanolic and aqueous extracts of the leaves have also been reported (Tonkal *et al*., 2005). The phytochemical composition of *Ziziphus spina- cristi* reported the presence of four saponin glycosides and alkaloids (Shahat *et al*., 2001 and Anthony, 2005).

Enterolobium contortisiliquum is an important species of the family Fabaceae. The essential oil of *Enterolobium contortisiliquum* seeds had been reported to have an antimicrobial activity (Shahat *et al*., 2008). The plant was reported to be rich in saponin (Mimaki *et al*., 2004), a substance responsible for molluscicidal activity (Hostettmann *et al.*, 1982, Osman *et al*., 2007).

It is now well established that in many plants the molluscicidal activity is due to the presence of saponin contents (Rawi *et al*., 1996, Osman *et al*., 2007 and Singh and Singh 2010) and alkaloid components (Melendez and Capriles, 2002, El-Ansary *et al*., 2003, Ahmed and Rifaat 2005, Silva *et al*., 2005 and Singh *et al*., 2010). According to these authors, plants containing one or more of these compounds are among the most promising for controlling schistosomiasis and fascioliasis.

Based on these facts and since *Euphorbia aphylla* (Euphorbiaceae), *Ziziphus spina*-christi (Rhamnaceae) and *Enterolobium contortisiliquum* (Fabaceae) have been described as plants rich in saponin and /or alkaloids. The present study is aimed to evaluate the molluscicidal activity of the ethanolic extracts of these plants against *Biomophalaria alexandrina* and *Lymnaea cailliaudi* (*nalatensis*) the snails' intermediate hosts of *Schistosoma mansoni* and *Fasciola* species respectively in a trial to open new areas of application of extracts of these plants as eco-friend molluscicides.

2. Materials and Methods: - Snails:

Laboratory bred uninfected adult *Biomphalaria alexandrina* snails (6-8mm in diameter) and *Lymnaea cailliaudi* (*nalatensis*) (8-10mm in shell length) from the stock reared in Medical Malacology Department, Theodor Bilharz Research Institute (TBRI) were used.

Plant Material:

The plants used in this study were *Euphorbia aphylla* (Family Euphorbiaceae), *Ziziphus spina-christi* (Family Rhamnaceae) and *Enterolobium contortisiliquum* (Family Fabaceae).The plant materials were collected locally from Faculty of Agriculture, Assiut University. Plant species was kindly identified and extracted by Prof. Dr. Zedan Z. Ibraheim, Pharmacognacy Department, Faculty of Pharmacy, Assiut University.Voucher specimens of each plant were kept in the Museum of Pharmacognacy Department, Faculty of Pharmacy.

Preparation of Plant Extracts:

The aerial parts of *Euphorbia aphylla*, Ziziphus *spina- christi* and the mature ripe fruit of *Enterolobium contortisiliquum* were cleaned, cut into small pieces and dried in shade then grounded using blender. About 250 g of air dried powdered plant material was extracted with ethanol (70%), filtered and distilled off under vacuum at temperature not exceeding 50 °C and the residues were stored in dry glass bottles (Bakry, 2009).

Preparation of Molluscicide Solutions:(According to Singab *et al*., 2006)

Stock solutions of 1000 ppm were freshly prepared by dissolving 1 g of each ethanolic extract in the minimal amount of dimethylsulfoxide (DMSO), and made up to 1000 ml by adding dechlorinated water. A series of concentrations (0.25 – 1000 ppm) were prepared from the stock solution of *Euphorbia aphylla* and double serial concentrations (100-200-400 etc.) were prepared from the stock solutions of *Ziziphus spina- christi* and *Enterolobium contortisiliquum*

Determination of molluscicidal activity:

WHO, 1965 guideline was followed for evaluation of the molluscicidal activity of the extracts.A series of exploratory experiments were conducted using the previously prepared concentrations to determine the toxicity range of the plant extracts against the tested snails. Once the extent of the toxicity range was determined, several intermediate concentrations were prepared from the stock solutions (diluted with dechlorinated water) to give mortalities between0-100% according to Osman *et al*., 2007.

For each experimental concentration three replicates were prepared, each of 10 snails/ L. Another three replicates were prepared in dechlorinated water as control. Snails were exposed to the molluscicide suspension for 24 hours at room temperature (exposure period). The tested snails were then left in water for another 24 hour and examined to assess mortality (Recovery period). Snails were considered dead if they probed and remained motionless or if the shell looked discoloured. Mortality rates were recorded. Probit regression analysis (SPSS version 7) aimed to determine the LC_{50} and LC_{90} values as well as their 95 % confidence limits were carried out according to Finney (1971).

3. Results

Molluscicidal activity of *Euphorbia aphylla*:

The effect of various concentrations of ethanol extract of the aerial portion of *Euphorbia aphylla* on adults of *Biomophalaria alexandrina* and *Lymnaea cailliaudi* (*nalatensis*)after 24 hour exposure are listed in tables 1and 2.

The LC_{50} and LC_{90} of this extract against *Biomophalaria alexandrina* after 24 hour exposure were 87.6 and 142.5ppm respectively. While the LC_{50} and LC_{90} of the same extract against *Lymnaea cailliaudi* (*nalatensis*) after 24 hour exposure were 0.66 and 0.88 ppm respectively.

There was a significant difference between molluscicidal activities of ethanol extract of *Euphorbia aphylla* against both snails. *Lymnaea cailliaudi* (*nalatensis*) were more sensitive to *Euphorbia aphylla* extract than *Biomophalaria alexandrina* adults.

The probit mortality showed that the response of the two snail species illustrated a linear relationship with the concentrations (dose ⁄ ppm) of the ethanol extract of *Euphorbia aphylla* as revealed in figure1, 2.The exposed snail species responded differently to different concentrations of the studied plant exract.

Molluscicidal activity of *Ziziphus spina christi*:

Molluscicidal effect of ethanol extract of the aerial portion of *Ziziphus spina- christi* on *B. alexandrina* gave negative results up to 1000ppm.

Molluscicidal effect of ethanol extracts of *Ziziphus spina christi* on *L. cailliaudi* showed LC_{50} 311ppm and LC_{90} 500 ppm. The probit mortality showed that the response of *L. cailliaudi* illustrated a linear relationship with the concentrations (dose ⁄ ppm) of the ethanol extract of *Zizyphus spina-christi* as revealed in figure 3.

Molluscicidal activity of *Enterolobium contortisilicuum*:

Ethanol extract of the fruit of *Enterolobium contortisilicuum* (Family Fabaceae) gave negative results against both snail species up to1000 ppm.

Table (1): Mortality rates, LC_{50} and LC_{90} of ethanolic extract of *Euphorbia aphylla* against *Biomophalaria alexandrina*

Conc. (ppm).	Number of tested snails	Number of dead snails	Mortality rates (%)	LC_{50}	LC_{90}
150	30	30	100	87.6(39.99–332.5)	142.5(98.3– 1092.3)
100	30	12	40		
50	30	9	30		
40	30	6	20		
20	30	0	0		

Probit Transformed Responses

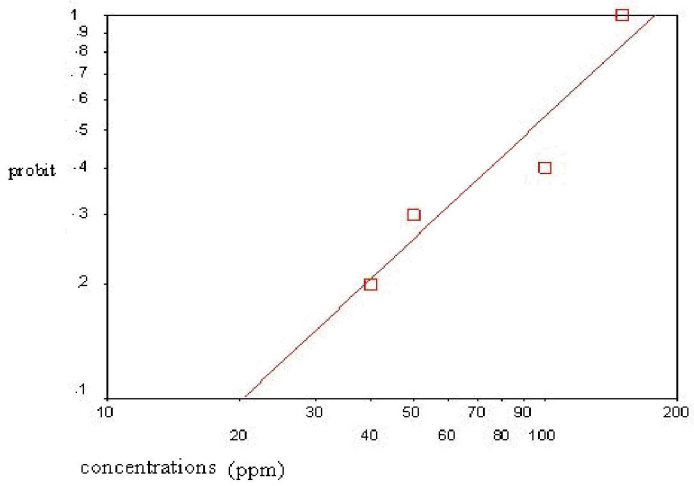

Figure 1.Dose/probit regression line of *Euphorbia aphylla* on *Biomophalaria alexandrina*

Table (2): Mortality rates, LC_{50} and LC_{90} of ethanolic extract of *Euphorbia aphylla* against *Lymnaea cailliaudi* (*nalatensis*).

Conc.(ppm)	Number of tested snails	Number of dead snails	Mortality rates (%)	LC_{50}	LC_{90}
1	30	30	100	0.66(0.62 - 0.70)	0.88(0.821-0.966)
0.80	30	24	80		
0.75	30	18	60		
0.60	30	12	40		
0.50	30	6	20		
0.25	30	0	0		

Probit Transformed Responses

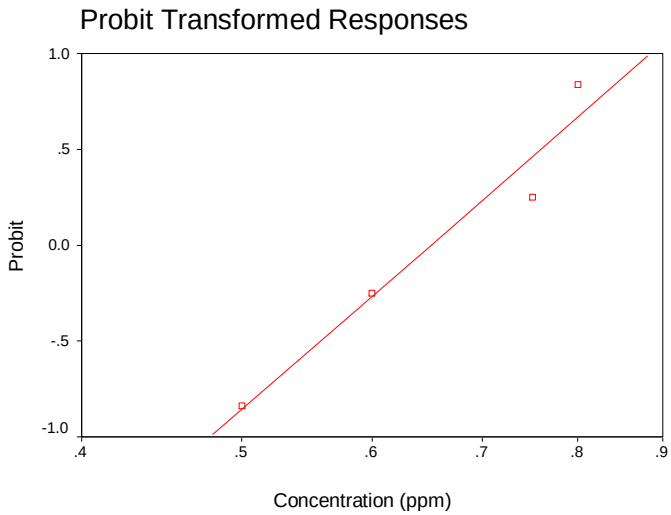

Figure 2. Dose/probit regression line of *Euphorbia aphylla* on *Lymnaea cailliaudi* (*nalatensis*)

Table (3): Mortality rates, LC_{50} and LC_{90} of ethanolic extract of *Ziziphus spina- christi* against *Lymnaea cailliaudi* (*nalatensis*)

Conc. (ppm)	Number of tested snails	Number of dead snails	Mortality rates (%)	LC_{50}	LC_{90}
800	30	30	100	311(163.83-465.68)	500(384.002 1089.676)
600	30	27	90		
400	30	24	80		
300	30	20	66.66		
200	30	5	16.66		
100	30	0	0		

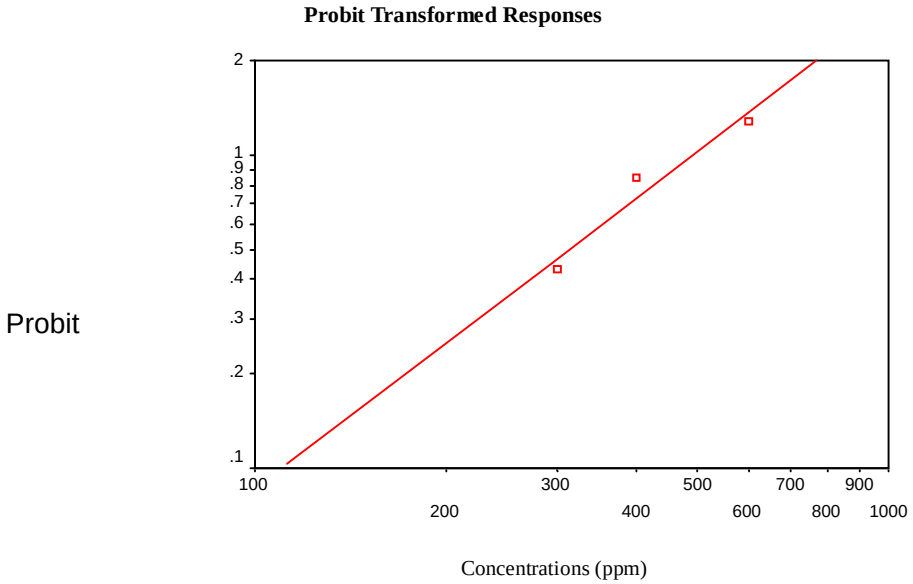

Figure 3.Dose/probit regression line of *Ziziphus spina-christi* on *Lymnaea cailliaudi* (*nalatensis*).

4. Discussion:

Schistosomiasis and fascioliasis are worldwide parasitic diseases infecting 207 and 17 million people respectively causing significant morbidity and mortality (WHO, 2006, 2010).

In Egypt, positive association between liver fluke infection and schistosomiasis was detected in several governorates. It was explained by the co-existance of both parasites intermediate hosts inhabiting the same type of water bodies(Haseeb *et al*., 2002).Once, these snails intermediate host destroyed, the life cycle will be disrupted (Hamed ,2010; Jigyasu and Sing, 2010). To achieve this goal, different synthetic molluscicidal compounds were used (Essawy *et al*., 2009;Kristoff *et al*., 2010).

The high costs of synthetic molluscicides, their toxicities to non-target organisms and even man as well as the complex organization required in their application, are a major setback to their continued use in schistosomiasis and fascioliasis control programmes. A potential cost effective alternative is the use of compounds from plant origin (WHO, 1993, 2003). Many plants have been screened for their intrinsic molluscicidal properties in an attempt to find an alternative to synthetic ones. Plants containing alkaloids and saponin are among the most promising plants for controlling schistosomiasis (El-Ansary *et al.* 2003, Silva *et al*. 2006; Singh and Singh, 2009; Singh *et al*., 2010).

Based on these facts and in view of extending problem of schistosomiasis and fascioliasis in terms of morbidity, mortality, treatment cost, it was decided to study three medicinal plant species namely;*Euphorbia aphylla*, *Ziziphus spina-christi*, and *Enterolobium contortisiliquum* for their molluscicidal activity against *Biomophalaria alexandrina* and *L. cailliaudi* (*nalatensis*) according to WHO, 1965 guidelines.

1) Molluscicidal activity of *Euphorbia aphylla*:

Euphorbiaceae is one of the largest families of

flowering plants. Members are widely distributed all around the world and some of which are yet to be identified (Sing and Sing, 2010). Molluscicidal activity is widespread in the family Euphorbiaceace, although activity varies greatly from species to species (Al-Zanbagi, 2005, Sharma *et al.*, 2009).

The present study demonstrated that the ethanol extract of *Euphorbia aphylla* possesses molluscicidal activity. These results are in harmony with Mello-Silva *et al*. (2006), Bakry (2009) and Sharma *et al.* (2009) who revealed the molluscicidal activity of different *Euphorbia* species with varying degrees of potency.

In the present study, The LC_{50} and LC_{90} values of *Euphorbia aphylla* against *B. alexandrina* are promising in comparison with some previously studied related plants as *Euphorbia gymnoclada* which did not show a molluscicidal effect against *B. glabrata* (Silva *et al*., 1971). *Euphorbia schimperiana* and *Euphorbia helioscopia* caused no mortality up to 100 ppm. on *Biomphalaria pfeifferi* (Al- Zanbagi 1999). Aqueous extract from *Jatropha curcas* L. (Euphorbiaceae) performed poorly against snails transmitting *Schistosoma mansoni* as 500 ppm caused 50% mortality (Rug and Ruppel, 2000).

Also this activity is better than *Atriplex stylosa*, *Guayacum officinalis* and *Calatropis procera* with LC_{90s} ranging from 180 to 360 ppm. against Egyptian *B. alexandrina*. On the other hand this activity is lower than that *E. splendens* (LC_{90} 73 and LC_{50} 40 ppm) (Bakry 2009). These differences in potency can be attributed to several factors including the locality of the plant species, time of collection of the plant sample, part used, storage conditions, method of extraction and solvents type (Brackenbury and Appleton, 1997 and Hassan *et al*., 2010).

The current study was extended to prove the molluscicidal effect of ethanol extract of *Euphorbia aphylla* on *L. cailliaudi* after 24 hours exposure. The LC_{50} and LC_{90} were 0.66 ppm and 0.88ppm respectively. This activity is higher than the latex of *E. hirta* against *Lymnaea acuminate* (LC_{50} 1.29 ppm) (Yadav and Singh, 2011). Also this activity is much higher than that of *Commiphora molmol* oil (LC_{50} and LC_{90} 50 and 85 ppm respectively) (Allam *et al.* 2001), *Phytolacca dodecandra* (Endod) (LC_{90} 2.8 ppm) (Yohannes *et al*., 1979) and *Meryta denhamii* (LC_{50} 26.4 and LC_{90} 70.8 ppm) (Hassan *et al.*, 2010) against *Lymnaea cailliaudi.*

It worth mention that *Commiphora molmol* (Myrrh) is a plant recommended as safe molluscicides(Massoud *et al*.,2004 , Al mathal and Fouad.,2006) and has been licensed for medical use in Egypt and several countries as a fasciolicidal and schistosomicidal drug with high efficacy and safety(Aly and Aly,2006 and Abdul-Samie *et al.*, 2010).Also *Phytolacca dodecandra* is the best studied plant molluscicide (Esser *et al*., 2003).

In the present study, *Lymnaea cailliaudi* has been found to be more susceptible than *Biomophalaria alexandrina* to the toxic action of *Euphorbia aphylla* with the latter requiring high concentrations as lethal doses when compared with the first species. This observation is in accordance with the findings of other investigators using other molluscicides (Allam *et al*., 2001 and Hassan *et al*., 2010). The difference in susceptibility of the two snails to the lethal effect of the same extract could be attributed to the natural resistance of different snail's genera and that the molluscicides may vary in their toxicological effects according to the species of the snails' used (Bakry and Hamdi, 2007 and Osman *et al*., 2007).

Beside its remarkable molluscicidal potency, *Euphorbia aphylla* also presents some very interesting characteristics for an ideal plant molluscicide. It is cosmopolitan and perennial plant. It is not edible to animals and easily cultivable (its multiplication is done by means of asexual reproduction which does not require frequent watering or application of pesticides or fertilizer) (Baptista *et al*., 1997 and Brickell, 2008).

2) Molluscicidal activity of *Ziziphus spina-christi*:

In the present study, the LC_{50} and LC_{90} of ethanol extract of *Ziziphus spina –christi* against *L. cailliaudi* after 24 hours exposure were 311and 500 ppm respectively. This activity is much higher than that reported for the Egyptian plant, *Ambrosia maritima* (damsissa) (LC_{90} 3000 ppm) against Egyptian *Lymnaea cailliaudi* (Abou Basha *et al*., 1994).

In the present study, based on the LC_{50} and LC_{90} values, *Ziziphus spina-christi* demonstrated less potent molluscicidal activity than *Euphorbia aphylla* against *Lymnaea cailliaudi* which can be attributed to the differences in each plant active ingredients, their mode of action and method of penetration of the snails (Rawi *et al*., 1996).

In the present study, *Ziziphus spina –christi* gave negative results up to 1000 ppm on *B. alexandrina*. These results reconfirmed that *Lymnae cailliaudi* is more sensitive. The possibility for the same plant extract to have molluscicidal activity against certain snail species and absence of activity against other species were recorded by Yasuraoka *et al*. (1980) who found that the seeds of *Jatropha carcas* (Family Euphorbiaceae) have a relatively high toxicity against *Oncomelania* while it showed no effect against *Lymnaea* snails.

3) Molluscicidal activity of *Enterolobium contortisiliquum*:

In the present study, *Enterolobium contortisiliquum* is selected due its richness in saponins (Mimaki *et al*., 2004). Saponins have haemolytic properties and toxic effect on most cold-blooded animals including snails and are proved to have molluscicidal activity (Herlt *et al*., 2002, Osman *et al*., 2007 and Singh and Singh, 2009).

In the present study, failure of *Enterolobium contortisiliquum* fruits to produce molluscicidal activity on both snail species up to 1000 ppm. could be attributed to the fact that saponins responsible for its activity are extracted in greater measures with more polar solvents. Supporting this explanation the results obtained by Hassan and Abdel-Rahman (2008) who found that the butanol fraction of *Hedera canariensis* (family Araliaceae) has molluscicidal activity against *Biomophalaria alexandrina* and *Lymnaea cailliaudi.* While ethyl acetate extract of the same plant was inactive. On the contrary, Hassan *et al* (2010) found that the butanol fraction of *Meryta denhamii* flowers which belongs to the same plant family was inactive and ethyl acetate was active against the same snail species.

Conclusion:

The use of *Euphorbia aphylla* may play vital role in controlling schistosomiasis and fascioliasis. The plant is commonly available, easy to collect and prepare for use. Therefore it is the most suitable for biological application which offers a potentially simple, readily available and inexpensive molluscicidal agent of plant origin. In future, more attention should be paid to the mechanism of action of *Euphorbia aphylla* on molluscs and application techniques for its use as plant molluscicides in rural communities. Phytochemical investigations to identify the bioactive ingredient(s) responsible for the molluscicidal potency are recommended. Toxilogical studies on man, fauna and flora of the fresh water are needed to conclude about the possible toxics properties of the ingredient(s).

Results of *Ziziphus spina- christi* suggests further laboratory tests to search for the presence of active component in the different parts o the plant. Such studies would increase their potential for future use as plant molluscicides.

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Perilesional Enhancement Assessment of Hepatic Metastases by Dynamic MRI

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Abstract: Evaluation of liver metastases is one of the most common indications for magnetic resonance (MR) imaging of the liver. In an era when contrast-enhanced CT and MRI are widely used for the assessment of focal liver lesions, peripheral rim enhancement of lesions on early phase images has been recognized as one of the characteristic findings of metastatic tumors. The purpose of this study is to determine whether perilesional parenchymal enhancement of hepatic metastases was correlated with the degree of tumoral enhancement on arterial phase images or tumor size using dynamic MRI. 67 hepatic metastases lesions in 33 patients were studied for perilesional enhancement and the study findings were high percent of perilesional enhancement at metastases compared to other histologic types of hepatic tumors like hepatocellular carcinoma and cavernous hemangioma. In addition, perilesional enhancement patterns observations showed it was the main component of rim enhancement rather than tumor hypervascular periphery that its size showed inverse correlation with the size of perilesional parenchymal enhancement. Characterizing the metastases from other hepatic tumors as well as different features of perilesional rim enhancement can influence therapeutic planning and an expectation of a better prognosis.
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Key words: perilesional • enhancement • MRI • dynamic • metastases

1. Introduction:

MRI is gradually emerging as the imaging modality of choice for detection and characterization of liver lesions. Identification of contrast enhancement pattern is crucial for characterization of hypervascular liver lesions [1].

Evaluation of liver metastases is one of the most common indications for magnetic resonance (MR) imaging of the liver. Metastases are the most common malignant liver lesions and are about 18–40 times more common than primary liver tumors [2].

In an era when contrast-enhanced CT and MRI are widely used for the assessment of focal liver lesions, peripheral rim enhancement of lesions on early phase images has been recognized as one of the characteristic findings of metastatic tumors [3].

Several reports with histologic correlation in metastatic tumors showed the growing tumor margin in the peripheral portion and the rather hypovascular components in the more central portion. Rim enhancement has been regarded as representing the hypervascular tumor periphery [3]. This rim enhancement has been explained by perilesional hepatic parenchymal enhancement around the tumor border [4].The main clinical impact of this type of perilesional enhancement is the potential for inaccuracies in tumor size estimation, which can have an influence on therapeutic planning and prognosis [5]. It has been established that complete surgical resection of liver metastases prolongs survival in eligible surgical candidates. Hence, detection, absolute quantification and localization of liver metastases are crucial as the findings alter the clinical outcome of the disease and patient management [6]. The purpose of this study is to determine whether perilesional parenchymal enhancement of hepatic metastases was correlated with the degree of tumoral enhancement on arterial phase images or tumor size using dynamic MRI.

2. Patients and Methods:

Patients

The study included 33 patients with known or suspected hepatic metastasis, 9 patients had histologic proof of primary extrahepatic lesions and liver lesions and 24 patients had histologic proof of primary extrahepatic lesions with definite serial increase in size of hepatic lesions.

The patients in this study were 19 men and 14 women; age range, 37–73 years; mean, 57 years ± 2SD . The primary cancer in the 33 patients with hepatic metastases was 13 colorectal cancers, 11 breast cancers, 5 pancreatic carcinomas, 2 lung cancers, one urinary bladder cancer and one renal cell carcinoma. 16 patients had single lesions, and 17 had multiple lesions.

All patients were examined with IV gadolinium chelate –enhanced dynamic MRI and all were analyzed for perilesional enhancement on arterial phase dynamic MRI in addition to unenhanced MR images.

Patients of this study were only patients success to meet the criteria for entry to this analysis, these criteria were identification of discrete focal lesions, successful arterial phase MRI that revealed contrast filling in intrahepatic portal veins without hepatic vein enhancement, no previous localized percutaneous ablation therapy for the focal hepatic lesions, and clearly visible lesions on unenhanced MR images for proper sizing of lesions before contrast media enhancement.

MRI

MRI was performed on a 1.5-T system (Signa, GE Healthcare) using a phased-array torso coil. T2-weighted imaging with the STIR turbo spin-echo technique (TR/TE, 3,500–4,000; inversion time, 65–80/165 msec; refocusing pulse, 130°; bandwidth, 325 Hz/pixel) was performed on the axial plane. After phase-contrast imaging with a double echo spoiled gradient-echo sequence (192/2.7 for opposed phase and 5.3 msec for in phase; flip angle, 80°; bandwidth, 488 Hz/pixel), Precontrast T1-weighted imaging and multiphase contrast-enhanced dynamic imaging were performed with the volumetric interpolated breath-hold examination (VIBE) with 3D spoiled gradient-echo sequences with fat suppression (.TR range/TE, 3–4/1.1; inversion time, 15 msec; flip angle, 15°; matrix, 256 x 128; section thickness, 10 mm; receiver bandwidth, 125 kHz; and number of signals acquired, one half. A 0.55 rectangular field of view was used to reduce the number of phase-encoding views[).]

For arterial phase imaging, all patients received a 20-25ml (1-2 mg/kg BW) of gadopentetate dimeglumine at a rate of 3 ml/sec through a catheter placed in a peripheral vein. Sequential spoiled gradient echo MR images were obtained through the lesion at 20 seconds, 60 and 120 seconds after injection.

Image Analysis:

For accurate measuring at the same level comparison and synchronization of the anatomic level of the arterial phase images with the precontrast images was done.

The tumor size was measured by using the longest dimension on axial images at precontrast images, then, comparing the precontrast images with arterial phase enhanced images to determine the presence of rim enhancement, perilesional enhancement was defined as circumferential high signal intensity around the lesions distinguished from the background hepatic parenchyma on contrast-enhanced images.

Every rim enhancement was measured as a whole by measuring the outer and inner diameters along the longest dimension for each lesion, after that, differentiate the size of thickness of circumferential perilesional enhancement from tumor vascularity in the periphery of each lesion by subtracting the unenhanced tumor size from the outer dimension of the rim enhancement to estimate the outer thickness of the rim enhancement (perilesional enhancement) and subtracting the inner dimension of the rim enhancement from the unenhanced tumor size to estimate the inner thickness of the rim enhancement that used to represent the tumor vascularity in the periphery of each lesion

Inner dimension of the rim was considered zero for diffuse enhancement lesions without any recognizable peripheral rim, perilesional enhancement was estimated in these lesions by subtracting the tumor size on the unenhanced image from the size of the lesion on the arterial phase images.

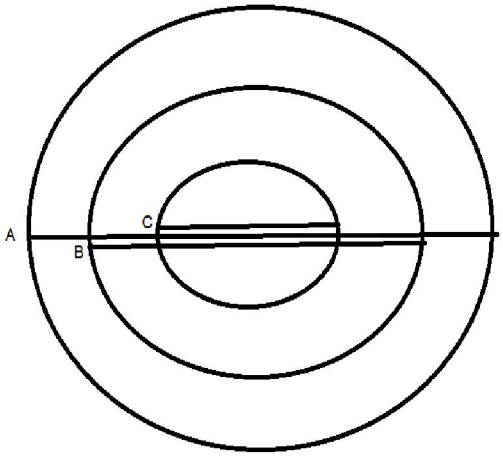
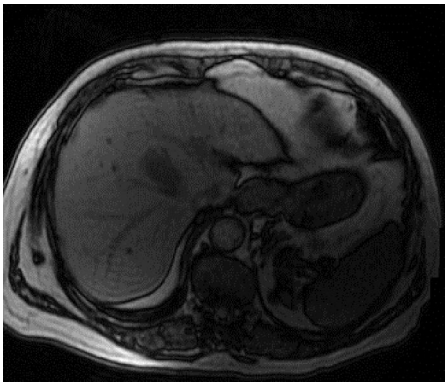
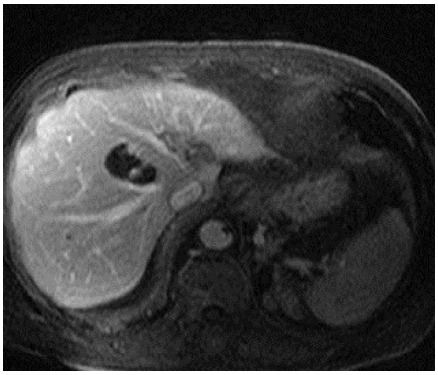


Fig. (1) Diagram for calculating thickness of perilesional enhancement and tumoral enhancement on arterial phase dynamic MR images. A line represents outer dimension of rim enhancement, B line represents tumor size on precontrast study and C line represents inner dimension of rim enhancement. Outer thickness of rim enhancement was estimated by subtracting B from A, and result was regarded as thickness of circumferential perilesional enhancement. Inner thickness of rim enhancement was estimated by subtracting C from B, and result used to represent tumor vascularity in periphery of each lesion.

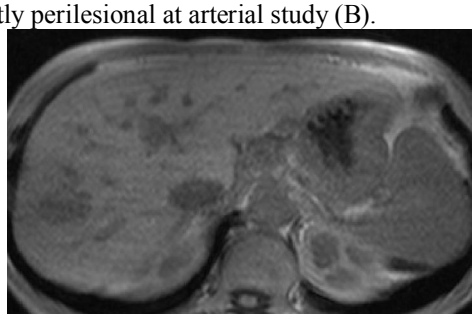


A

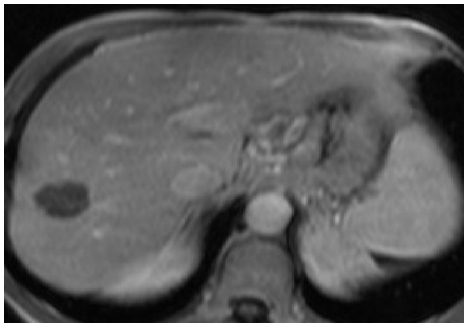


B

Fig. (2): 57 years old man with hepatic metastasis from known colon cancer. Precontrast study (A) shows low signal intensity with rim enhancement mostly perilesional at arterial study (B).

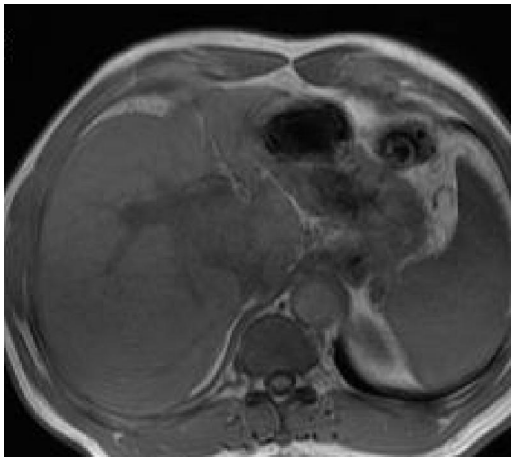


A

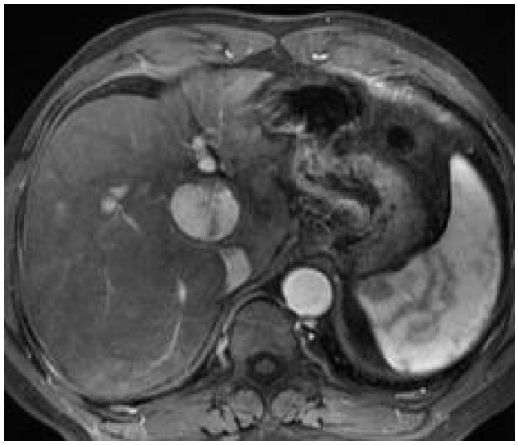


B

Fig. (3) 63 years old man with hepatic metastasis from known colon cancer. Precontrast study (A) shows low signal intensity with rim enhancement mostly perilesional at arterial study (B).



A



B

Fig. (4): 53-year-old man with metastases from colon cancer. Precontrast study (A) shows low-signal-intensity lesion in liver with diffuse enhancement at arterial phase (B).

3. Results

The study included 33 patients showed 67 hepatic metastatic lesions.

42 lesions (about 63%) from the all 67 lesions showed hyperintense rim enhancement distinguished from the surrounding liver on arterial phase dynamic MR images with the thickness of rim enhancement calculated by the difference between the outer dimension and the inner dimension of the rim enhancement ranged from 2 to 14 mm (mean, 6 mm). the longest dimension of most of these lesions (39 from 42 lesions, 92 %) showed more than 2mm increase in size at arterial phase image when compared with the unenhanced images.

9 lesions (about 13%) showed diffuse enhancement of the entire lesion on the arterial phase dynamic MR images. The size of the hyperintensity on the arterial phase images was larger than that measured on the unenhanced T1-weighted images,

the difference between the 2 sizes represent perilesional enhancement.

16 lesions (about 24%) showed no rim enhancement on arterial phase dynamic images.

The 67 lesions demonstrated variable sizes ranged from 10 -112mm with mean 24mm. 4 lesions from the 16 lesions with no rim enhancement on arterial phase dynamic study showed more than 2 mm reduction in diameter on the arterial phase dynamic images, suggesting the presence of peripherally increased tumor vascularity with isointense contrast enhancement indistinguishable from the surrounding hepatic parenchyma. The other 12 lesions showed less than a 2mm reduction in diameter.

Size of lesions with hyperintense rim enhancement (n = 42; 2 5± 18 [SD] mm) showed no significant difference with lesions without distinguishable rim enhancement (n = 16; 26 ± 22 mm) (p > 0.05). For the 42 lesions with rim enhancement, the thickness of the outer portion of the rim enhancement (mean, 6 ± 3 mm) was significantly larger than that of the inner portion (mean, 1 ± 2 mm) (p < 0.001), which suggested that the main component of the rim enhancement was perilesional enhancement rather than tumoral enhancement. In these cases, the tumor size was not correlated with the thickness of the rim enhancement (p > 0.05).

However, the degree of tumoral enhancement (inner thickness of the rim enhancement) showed a significant inverse correlation (r = -0.389) with the thickness of the perilesional rim enhancement (p < 0.001).

4. Discussion:

Previous studies observations supposed rim enhancement was mainly shown at the extralesional area [7] and showed a positive correlation with the degree of histologic changes of the extralesional tumor border, including desmoplastic reaction, inflammation, or vascular proliferation [11].

From our study observations, the main component of rim enhancement might be extralesional and partly may represent the hypervascular tumor periphery portion during the arterial phase dynamic MRI.

One of the main observations in this study is the lesions with a higher vascular component showed a lesser degree of perilesional enhancement, and the lesions with a lower vascular component showed a greater degree of perilesional enhancement, this observation differs from the results of previous investigations involving other types of tumors, including hepatocellular carcinoma or hepatic cavernous hemangioma, (Ueda *and others 2006)*[9] reported that perilesional corona enhancement was observed after complete filling of hypervascular hepatocellular carcinoma in their single level CT arteriography study. Moreover, in a report by (Yu *and others 2002)*[10] with dynamic MRI, perilesional enhancement tended to be found in larger and more hypervascular tumors in hepatocellular carcinomas.

Another important observation at this study was the high incidence of perilesional enhancement in hepatic metastases (63 %) comparing to overall incidence of perilesional enhancement for uncomplicated hepatocellular carcinoma without gross portal vein invasion with a value of less than 12% (10).

(Terayama *and others 2002)*[11] in a study of single-level CT hepatic arteriography with pathologic correlation reported hypovascular metastases tended to show early appearance of rim enhancement and hypervascular metastases showed more delayed rim enhancement. They proposed that the rim enhancement of hepatic metastases is caused by altered hemodynamics in the surrounding liver parenchyma for hypovascular lesions in addition to the drainage flow from the hypervascular lesions.

Observation in this study was the reverse correlation between the thickness of the perilesional enhancement and the hypervascular area of the tumor.

In addition to characterizing the metastases from other hepatic tumors, these different features of perilesional rim enhancement can influence therapeutic planning and an expectation of a better prognosis, the area of perilesional rim enhancement is open to the possibility of microscopic tumor cell infiltration, and we can justify the widening of safety margin for local ablation therapy or partial hepatic resection.

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The Ethmoidal Ganglion and its Anatomical Relations in Lacertilia (Squamata: Reptilia)

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Abstract: The ethmoidal ganglion of three lizards belonging to three different families is described. Such ganglion is represented by one part in both *Uromastyx aegypteus* (Family: Agamidae) and *Varanus griseus griseus* (Family: Varanidae) and by two parts in *Sphenops sepsoides* (Family: Lacertidae). In *Uromastyx* and *Varanus* the ganglion lies on the ramus palatinus of the nervus facialis. In *Sphenops*, a small (ventral) part of the ganglion is located on the ramus palatinus while its main (dorsal) part is found on the anastomosing branch between the rami palatinus facialis and nasalis of the nervus trigeminus. In the present study, the ganglion is found at an anastomosing branch between the ramus palatinus of the nervus facialis and the ramus nasalis of the nervus trigeminus in *Sphenops*. In *Varanus* it is found where the ramus palatinus becomes very close to the ramus nasalis, whereas, in *Uromastyx* the ganglion is located posterior to the origin of the anastomosing branch.
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Keywords: Ethmoidal ganglion – *Uromastyx*, *Varanus* and *Sphenops*.

Introduction

The ethmoidal ganglion which have received various names (orbitonasalis, sphenoethmoidal, sphenopalatine) from different authors is not found in all reptiles. It is regularly found in lizards and snakes and it is lacking in Chelonia and Crocodilia (Shiino, 1912 & 1914, Ogushi, 1913 a & b and Soliman, 1964).

In Reptilia, the ethmoidal ganglion is located at the place of anastomosis of the ramus nasalis of the nervus trigeminus and the ramus palatinus of the nervus facialis (Gaupp, 1888; Bellairs, 1949). Willard (1915) and Oelrich (1956) gave detailed study of the ganglion of *Anolis carolinensis* and *Ctenosaura* respectively. Soliman (1968) and Hegazy and Mostafa (1990) studied this ganglion in some lizards. Mostafa (1991) presented a study for the ganglion in three colubrid snakes.

It is obvious from the literature cited that, there is a little information about the structure, nature, position and the relations of the ethmoidal ganglion. In addition there are a few descriptive studies for the ethmoidal ganglion in relation to the great size of class Reptilia. So, the study of this subject is necessary both morphologically and phylogenetically.

2. Materials and Methods

Three lacertilian species belonging to three different families were choosen for this study; *Uromastyx aegyptius* (Family: Agamidae), *Sphenops sepsoides* (Family: Lacertidae) and *Varanus griseus griseus* (Family: Varanidae).

Uromastyx aegyptius is also known as spiny-tailed lizard or dabb lizard. This species inhabits hard sand and gravel desert, preferring flat areas with light vegetation. It is primarily herbivorous, but occasionally eats young insects. It digs deep burrows in the hard soil usually with single enterance. It is a diurnal species spending most of its walking hours basking in the sun near burrow entrance especially at morning time. This species is adapted to the arid habitat. It is found throughout the North Africa, Middle east across south-central Asia and into India. Female dobb lizards can lay from 5 to 40 eggs. The eggs are laid (at July – August) approximately 30 days after copulation with an incubation time of 70-80 days.

The embryos of *Uromastyx* of this study were collected from Gabal Al-Maghara about 80-90 km south of El-Arish city, Northern Sinai, as fertilized eggs during the last days of the incubation period (at August). After careful removing of the embryos from the shells, they were fixed very soon in an aqueous Bouin's fluid for 24 hours.

Sphenops sepsoides is a sand dwelling fossorial species with extremely reduced limbs for sand locomotion. It is found in a wide variety of habitats; ranging from depressions of Western desert to the sand spots in the rock wadies of the eastern desert and Sinai. It appears to be nocturnal and feeds entirely on fossorial insects (i.e., insectivore), this species is ovoviviparous.

The embryos of *Sphenops sepsoides* are collected from two pregnant females in the lab. The fully formed embryos were soon fixed in aqueous Bouin's fluid for 24 hours.

Varanus griseus griseus is a large diurnal lizard. It feeds on lizards, snakes, and rodents. This species lives in sandy areas throughout the Western and

Eastern deserts and Northern of Sinai. It is found in North Africa and Western Asia. The desert monitor lizard move in groups on searching for food.

Ten youngs (newly hatched) *Varanus* were collected from Romana territories at El-Arish city, Northern of Sinai. These young, after anaesthetizing, were fixed in aqueous Bouin's fluid for 36 hours.

After fixation, the specimens of both *Uromastyx* and *Varanus* were passed into decalcificating EDTA solution for about 30 to 50 days. Then washed in distilled water and transferred to 70% ethyl alcohol for 48 hours. The embryos were embedded in paraffin wax then serially transversely sectioned at 10 µm thickness in *Uromastyx* and 15 µm in both *Sphenops* and *Varanus*. The serial sections were then stained in Mallory's triple stain (Pantin, 1946) for *Uromastyx* and *Varanus* and in haematoxylin and then counter stained with eosin for *Sphenops*.

The serial sections were drawn by the projector. From the projection the graphic reconstructions of the ciliary ganglion were made. Photomicrographs for parts of the transverse sections were provided to elucidate the position and relations of the ganglion to the other structures of the head.

3. Results

The microscopic investigation of the serial sections of the three lizards studied, *Uromastyx aegyptius*, *Sphenops sepsoides* and *Varanus griseus griseus*, revealed the presence of the ethmoidal ganglion as a collection of ganglionic cells in the most anterior orbital (orbitonasal) region of the head. In both *Uromastyx aegyptius* (Figs. 1 & 7, G.ET.) and *Varanus griseus griseus* (Figs. 2 & 4, G.ET.), the ganglion is represented by one part. However, in *Sphenops sepsoides*, it is formed of two parts (Figs. 3, 8 & 9, G.ET.DP. & G.ET.VP.).

In *Uromastyx aegyptius*, the ethmoidal ganglion is elliptical in shape as it appears in the transverse serial sections (Figs. 1 & 7, G.ET.). It measures about 60 µm in its length. In *Varanus griseus griseus*, it appears as an irregular mass of ganglionic cells, which measures about 252 µm in its length (Figs. 4 & 5, G.ET.). In *Sphenops sepsoides*, however, the ethmoidal ganglion appears as two parts; one small and ventral (Figs. 3 & 9, G.ET.VP.) and the other large and dorsal (Figs. 3 & 8, G.ET.DP.). The ventral part measures about 60 µm in its length. The dorsal part is large.

In both *Uromastyx aegyptius* (Figs. 1 & 7) and *Varanus griseus griseus* (Figs. 2, 4 & 5), the ganglion lies totally along the course of the ramus palatinus of the nervus facialis. In *Uromastyx aegyptius*, it lies shortly posterior to the origin of the anastomosing branch between ramus palatinus and the ramus nasalis of the nervus trigeminus (Figs. 1 & 6, R.CM.NA. + PAM.VII). The microscopic examination of the serial sections fails to fined any ganglionic cells either in the communicating branch or on the ramus nasalis. In *Varaus griseus griseus*, the ethmoidal ganglion (Figs. 2, 4 & 5) is found along the course of the palatine ramus, where it becomes closely applied to the ramus nasalis in the orbitonasal region of the head.

In *Sphenops sepsoides*, the lower or ventral part of the ganglion (Figs. 1 & 9, G.ET.VP.) lies along the ramus palatinus of the nervus facialis, whereas it dorsal part (Figs. 3 & 8, G.ET.DP.) lies along the communicating branch arising from the dorsal surface of the ventral part of the ganglion to anastomose with ramus nasalis (Figs. 3 & 8, R.CM.NA. + PA.VII).

Regarding the position of the ganglion in the three lizards studied, it has been found that, the ethmoidal ganglion is located in the most anterior orbital (orbitonasal) region of the head. In *Uromastyx aegyptius* (Fig. 7) the ganglion lies dorsal to the vomer bone (V) and ventrolateral to the inferior oblique muscle of the eye (M.OIF.). In *Varanus griseus griseus* (Fig. 4). The ganglion is located dorsal to the planum anteorbitale (PAOR.), ventral and ventromedial to both the ramus nasalis (R.NA.V) and the fenestra olfactoria evehens (F.OL.EV.) and medial to the orbital sinus (OR.SI.). In *Sphenops sepsoides* (Figs. 8 & 9), it is located ventral to the taenia marginalis anterior (TMA.), ventromedial to the ramus nasalis (R.NA.V), dorsomedial to the planum anteorbitale (PAOR.) and lateral to the interorbital septum (IOR.S.).

4. Discussion

In the three species studied, a well developed ethmoidal ganglion is found at an anastomosis between the ramus palatinus of the nervus facialis and ramus nasalis of the nervus trigeminus in the orbitonasal region in both *Varanus griseus griseus* and *Sphenops sepsoides*. In *Uromastyx aegyptius*, it is located shortly posterior to the origin of the anastomosing branch. The ganglion gives off a dorsal anastomosing branch which connects with the ramus nasalis of the nervus trigeminus in *Sphenops sepsoides*, whereas in *Varanus griseus griseus* the ramus palatinus extends closely applied to the ramus nasalis at the position of the ganglion along the former ramus. It is composed of a single part which is closely applied to the ramus palatinus of the nervus facialis in both *Uromastyx aegyptius* and *Varanus griseus griseus*, while in *Sphenops sepsoides*, it is composed of two parts one ventral applied to the ramus palatinus and a dorsal one found on the anastomosing branch. The result observed in both *Uromastyx* and *Varanus* is similar to what was described by Soliman (1968) in *Mabuya quinquetaeniata*, Hegazy and Mostafa (1990) in *Agama sinaita* and by Dakrory (1994) in the amphisbaenian *Diplometopon zarudnyi*. The same

condition was also observed in the snakes *Cerastes vipera* (Hegazy, 1976), *Spaleosophis diadema* (Mostafa, 1990 and 1991) and in *Natrix tessellate* (Dakrory and Mahgoub, 2004).

The location of the ethmoidal ganglion in *Varanus griseus* is typical to that observed by Watkinson (1906) in *Varanus bivittatus*. In this respect, the location of the ethmoidal ganglion, wherever, shows a wide variation in Lacertilia. In this study, the ganglion in question, is closely applied to the ramus palatinus in *Uromastyx aegyptius* and *Varanus griseus griseus*. The ganglion is closely related to the ramus nasalis, and it is in connection with the medial palatine ramus in *Anolis carolinensis* (Willard, 1915), *Lacerta viridis*, *Ptyodactylus hasselquistii* and *Acanthodactylus boskiana* (Soliman, 1968), *Chalcides ocellatus* (Soliman and Hegazy, 1969), *Stenodactylus slevini* (Hegazy and Mostafa, 1990) and *Acanthodactylus opheodurus* (Mostafa, 1990). On the other hand, the ganglion is found in the middle of the anastomosing branch which connects the rami nasalis and medial palatine in *Ctenosaura pectinata* (Oelrich, 1956), *Agama mutabilis* (Soliman, 1968) and *Eumeces schneiderii* (Hegazy and Mostafa, 1990). In the Ophidia, the ganglion is composed of one part that is closely applied to the ramus palatinus in *Natrix tessellate* (Dakrory and Mahgoub, 2004).

The condition found in *Sphenops sepsoides* studied was observed in many lizards and snakes but with slight disagreement, as they have been studied. Between the two parts of the ganglion is found a communicating branch. In *Tarentola mauritanica* (Soliman and Mostafa, 1984) and *Ptyodactylus hasselquistii* (Abdel-Kader, 1990), one part of the ganglion is closely applied to the ramus nasalis, whereas the other part is close to the medial palatine ramus. In *Agama pallida* (Abdel-Kader, 1990); one part of the ganglion is close to the medial palatine ramus and the other lies at the middle of the anastomosing branch.

Concerning the matter in snakes, the two parts of the ganglion are closely applied to the medial palatine ramus in *Psammophis sibilans* (Hegazy, 1976) and in *Coluber elegantissimus* (Mostafa, 1991). In *Eryx jaculus* (Hegazy, 1976), one part is applied to the ramus nasalis and the other to the medial palatine ramus. In *Psammophis schokari* (Mostafa, 1991) one part is entirely located in the medial palatine ramus and the other on the communicating branch.

Apart from Squamata, the ganglion ethmoidale, on the other hand, has not been found in Chelonia (Shiino, 1912; Ogushi, 1913 a & b; Soliman, 1964). Similarly, in Crocodilia; Shiino (1914) neglects any sort of connection between the rami palatinus and nasalis anteriorly in the orbital region and hence mentioned nothing about the ganglion.

In Amphibia, early works of Coghill (1902) and Norris (1908) show the presence of the ethmoidal ganglion in *Amblystoma tigrinum* and *Amphiuma means*, respectively. Again, the anastomosis between the ramus ophthalmicus profundus of the nervus trigeminus and the ramus palatinus of the nervus facialis was found in *Salamandra maculosa* (Gaupp, 1888); *Amblystoma punctatum* (Herrick, 1894), *Siren lacertian* (Norris, 1913) and in *Xanopus laevis* (Paterson, 1939).

In birds, an anastomosis between the ramus ophthalmicus profundus of the nervus trigeminus and the ramus palatinus of the nervus facialis was described by Cords (1904) and Haler von Hallerstein (1934). Stresemann (1927) described the ethmoidal ganglion in birds, he recorded its lacking in *Struthio*. Soliman *et al*. (1986) described the so called sphenopalatine ganglion from *Passer domesticus*, *Upupa epops* major and *Streptopelia senegalensis aegyptiaca*.

In Pisces; Freihofer (1978), Dakrory (2000) and Ali and Dakrory (2008) neglected completely the presence of this ganglion.

Regarding the function of the ethmoidal ganglion, Stresemann (1927) mentioned that there is an exchange of fibres between the ramus palatinus of the nervus facialis and both the nervus glossopharyngeus and the parasympathetic nerves, and that the ganglion ethmoidale is formed in the region of exchange of fibres. Norris (1908) stated that there seems to be a ganglion on the ramus palatinus shortly before the anastomosis. Beyond the anastomosis, he found mixed cutaneous and viscerosensory rami. Such observation agrees with that of Willard (1915) which shows that the ganglion ethmoidale is more closely related to the ramus palatinus of the nervus facialis.

As to the nature of the ethmoidal ganglion, Carpenter (1906) gave evidence about its sympathetic character. Again Willard (1915) on his dissection of this ganglion and its connections in *Anolis carolinensis* showed that it has to do largely with non-medullated fibres, and that the nature of its cells shows its sympathetic character.

From the above mentioned discussion, the authors can conclude that, in many features, the ethmoidal ganglion on Lacertilia is parallel to Ophidia. This suggests that theory of considering the Squamata (Lacertilia and Ophidia) as modern advanced reptiles, whereas representatives of the Chelonia occupy an early side branch of the stem Reptilia. Moreover, the phylogenetic variation of the ganglion ethmoidale in vertebrates suggests that the lizards (Squamata) stand half-way between the fishes and Amphibia from one side and the Aves from the other side, as to its evolution.

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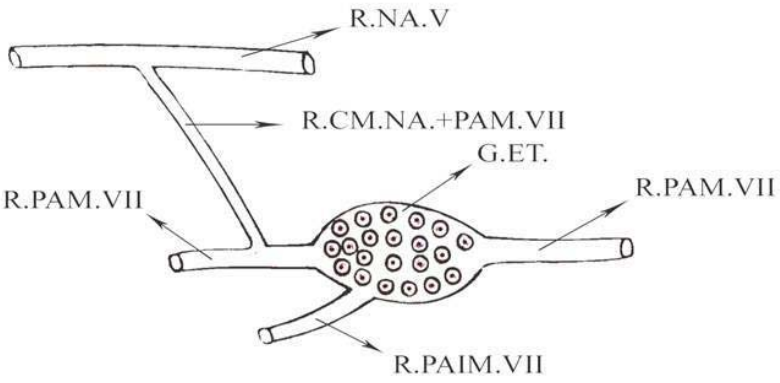

Fig. 1: Graphic reconstruction of the ethmoidal ganglion of *Uromastyx aegyptius* in a lateral view. GE.T., Ethmoidal ganglion, R.CM.NA.+ PA.VII, Ramus communicanus between the ramus nasalis of the nervus trigeminus and the ramus palatines, R.NA.V, Ramus nasalis of the nervus trigeminus, R.PAIM.VII, Ramus palatinus intermedialis of the nervus facialis, R.PAM.VII, Ramus palatinus medialis of the nervus facialis.

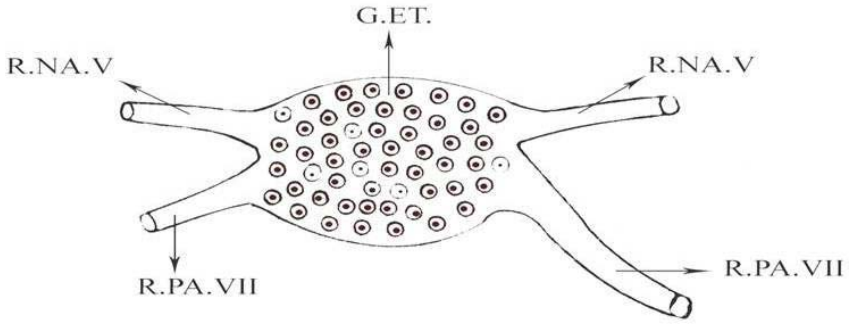

Fig. 2: Graphic reconstruction of the ethmoidal ganglion of *Varanus griseus* in a lateral view. GE.T., Ethmoidal ganglion, R.NA.V, Ramus nasalis of the nervus trigeminus, R.PA.VII, Ramus palatinus of the nervus facialis.

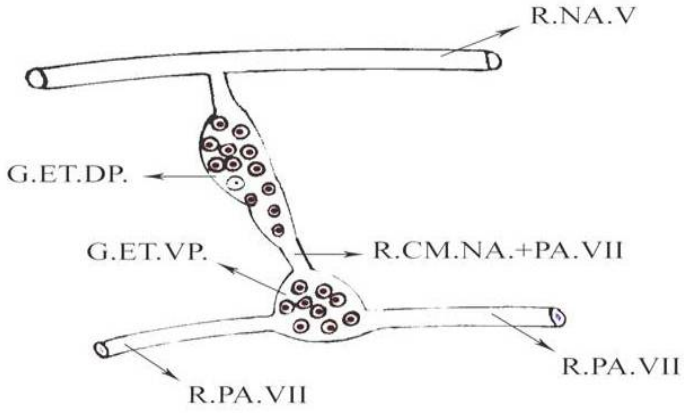

Fig. 3: Graphic reconstruction of the ethmoidal ganglion of *Sphenopus sepsoides* in a lateral view. G.ET.DP., Dorsal part of the ethmoidal ganglion, G.ET.VP., Ventral part of the ethmoidal ganglion, X40. R.CM.NA.+ PA.VII, Ramus communicanus between the ramus nasalis of the nervus trigeminus and the ramus palatinus R.NA.V, Ramus nasalis of the nervus trigeminus, R.PA.VII, Ramus palatinus of the nervus facialis

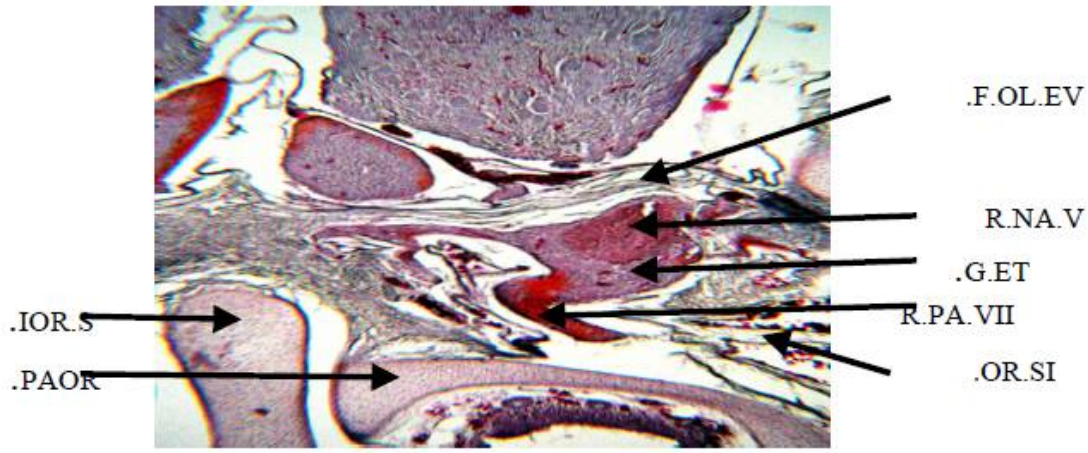

Fig. 4: A photomicrograph of a part of transverse section of *Varanus griseus gresius* showing the position of the ethmoidal ganglion. X40. F.OL.EV., Foramen olfactorium evehens, G.ET., Ethmoidal ganglion, IOR.S., Interorbital septum, OR.SI., Orbital sinus, PAOR., Planum anteorbitale, R.NA.V, Ramus nasalis of the nervus trigeminus, R.PA.VII, Ramus palatinus of the nervus facialis.

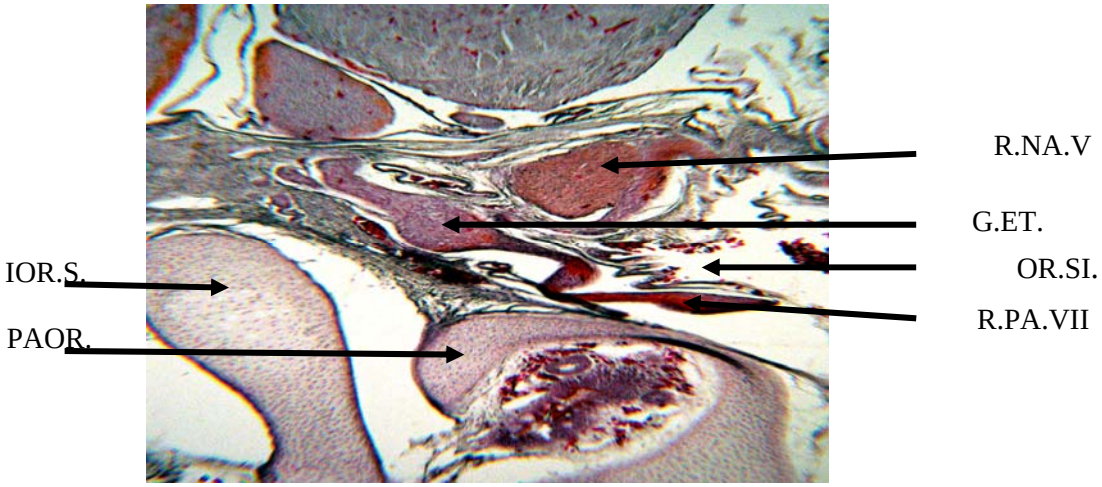

Fig. 5: A photomicrograph of a part of transverse section of *Varanus griseus gresius* showing the relation of the ethmoidal ganglion to both the rami palatinus facialis and nasalis of the nervus trigeminus. X40. G.ET., Ethmoidal ganglion, IOR.S., Interorbital septum, OR.SI., Orbital sinus, PAOR., Planum anteorbitale, R.NA.V, Ramus nasalis of the nervus trigeminus, R.PA.VII, Ramus palatinus of the nervus facialis.

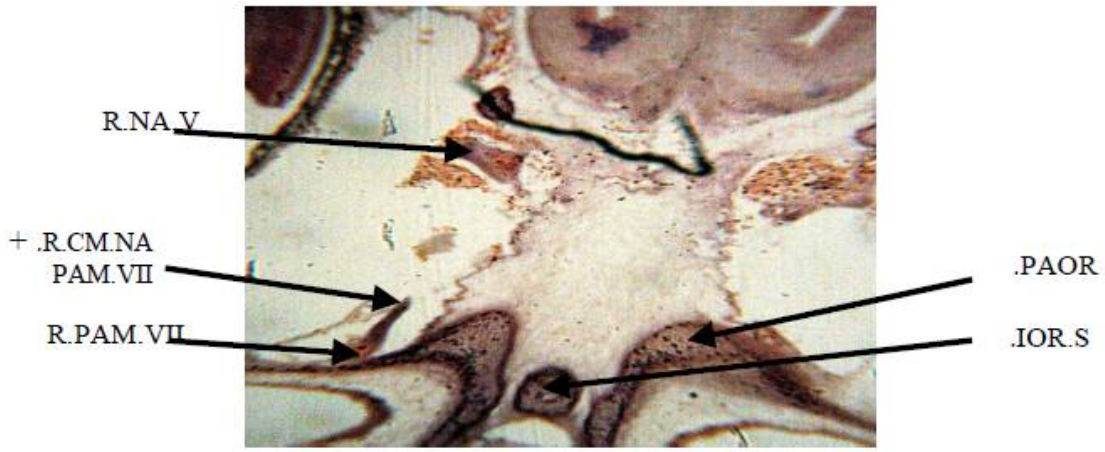

Fig. 6: A photomicrograph of a part of transverse section of *Uromastyx aegyptius* demonstrating the origin of the ramus communicanus between the ramus palatinus medialis of the nervus facialis and the ramus nasalis of the nervus trigeminus. X40. IOR.S., Interorbital septum, OR.SI., PAOR., Planum anteorbitale, R.CM.NA.+ PAM.VII, Ramus communicanus between the ramus nasalis the nervus trigeminus and the ramus palatinus medialis, R.NA.V, Ramus nasalis of the nervus trigeminus, R.PAM.VII, Ramus palatinus medialis of the nervus facialis**.**

Fig. 7: A photomicrograph of a part of transverse section of *Uromastyx aegyptius* illustrating the position of the ethmoidal ganglion and the origin of the ramus palatinus intermedialis of the nervus facialis. X40. G.ET., Ethmoidal ganglion, IOR.S., Interorbital septum, M.OIF., Inferior oblique muscle, M.OS., Superior oblique muscle, R.NA.V, Ramus nasalis of the nervus trigeminus, R.PAIM.VII, Ramus palatinus intermedialis of the nervus facialis, R.PAM.VII, Ramus palatinus medialis of the nervus facialis. V., Prevomer.

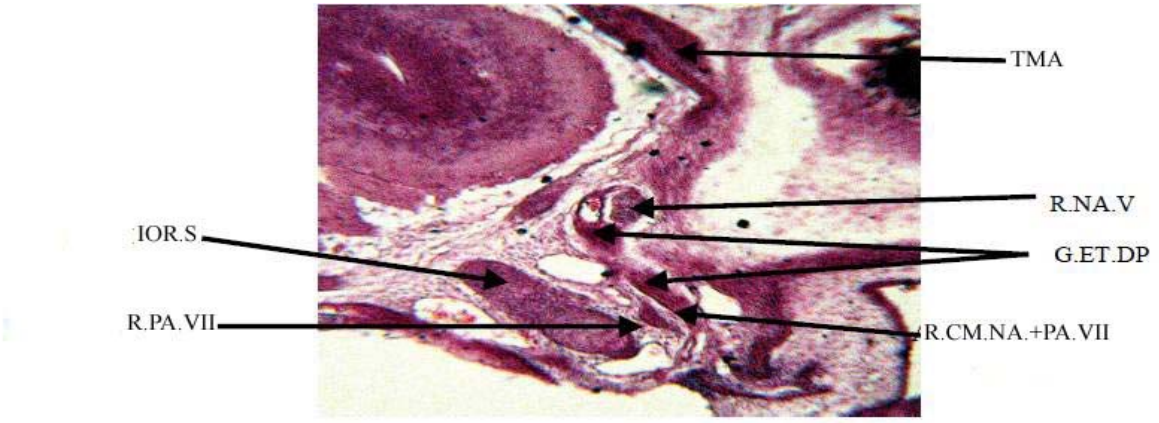

Fig. 8: A photomicrograph of a part of transverse section of *Sphenops sepsoids* showing the position of the dorsal part of the ethmoidal ganglion and the ramus communicanus between the ramus palatinus facialis and ramus nasalis of the versus trigeminus. X40. G.ET.DP., Dorsal part of the ethmoidal ganglion, IOR.S., Interorbital septum, R.CM.NA.+ PA.VII, Ramus communicanus between the ramus nasalis of the nervus trigeminus and the ramus palatines, R.NA.V, Ramus nasalis of the nervus trigeminus, R.PA.VII, Ramus palatinus of the nervus facialis, TMA., Taenia marginalis anterior.

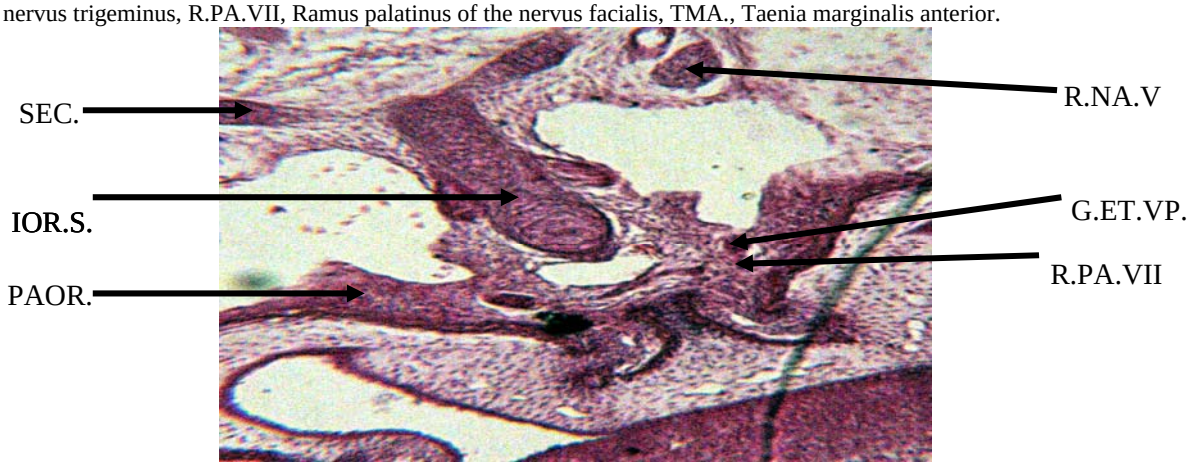

Fig. 9: A photomicrograph of a part of transverse section of *Sphenops sepsoids* demonstrating the position of the ventral part of the ethmoidal ganglion and the ramus palatinus of the nervus facialis. X100. G.ET.VP., Ventral part of the ethmoidal ganglion, IOR.S., Interorbital septum, PAOR., Planum anteorbitale, R.NA.V, Ramus nasalis of the nervus trigeminus, R.PA.VII, Ramus palatinus of the nervus facialis, SEC., Sephenoethmoidal commissure.

Patient Education: its Effect on Qualiy of Life of Patients with Cancer on Chemotherapy

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Abstract: Background: Cancer is a devastating disease that can negatively affect a patient's quality of life. Treatment for malignancies in particular, frequently involves complex, intense, and prolonged chemotherapy regimens. Aim of the Work: to assess the effect of educational intervention on quality of life for patients with cancer on chemotherapy. Subjects and Methods: This quasi-experimental study was conducted at the Radiotherapy and Nuclear Medicine Department affiliated to Ain-Shams University Hospitals, on a purposive sample of fifty patients recruited into this study. The inclusion criteria were adult patients, their age ranged between 20-50 years, on chemotherapy; received at least 2-3 sessions of chemotherapeutic agent. The tools included interviewing questionnaire sheet and Quality of Life Index Scale consisting of four dimensions: Physical, psychological, social and spiritual. The educational intervention was conducted through five sessions each one lasted from 30-45 minutes. Effects of the educational intervention on patient's condition investigated done through comparing the pre, post one month and post six months assessment of the patients regarding their level of knowledge and quality of life. Results: There is highly statistically significant difference in patient's level of knowledge pre, post one month and post six months from implementation of the educational intervention about cancer regarding to all items. Comparison of patient's quality of life dimensions pre, post one month and post six months from implementation of the educational intervention revealed there is highly statistically significant differences among them (77.78 ±6.54, 63.54 ±7.85& 50.24±5.47 respectively at $p<0.001$). Conclusion and Recommendations: Education has highly statistically significant positive effect in increasing the level of patients' knowledge and improving their quality of life. Further investigation could be undertaken to document the cost effectiveness of educational effects in reducing hospital stay, decreasing readmission, improving the personnel quality of life and minimizing complications of illness and therapies.
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Key word: Cancer, quality of life, educational intervention

1. Introduction

Cancer is a group of more than 200 diseases characterized by uncontrolled and unregulated growth of cells. It can occur in persons of all ethnicities and is a major health problem. The word cancer is viewed as being synonymous with death, pain, and disfigurement. However, attitudes toward cancer do not fit today's status of the treatment and control of cancer. Cancer is a synonymous with certain terms such as malignant neoplasm, tumor, malignancy, carcinoma, and aberrant cell growth (**Coleman, 2002).**

Cancer emerges as a public health problem around the world. Each year, it affects more than 10 million people and killed 7.6 million people all over the world (around 13% of all deaths in 2008).More than 70% of all cancer deaths occurred in low- and middle-income countries. Deaths from cancer worldwide are projected to continue to rise to over 11 million by year 2030. (**World Health Organization, WHO, 2011**).

Quality of life (QOL) is defined as individual perception of life, values, objectives, standards, and interests in the framework of culture. QOL is increasingly being used as a primary outcome measure in studies to evaluate the effectiveness of treatment. Patients generally instead of measuring lipoprotein level, blood pressure, and the electrocardiogram, make decisions about their health care by means of QOL which estimates the effects on outcomes important to themselves. (**Timby & Smith, 2007**).

An increasingly important issue in oncology is to evaluate QOL in patient with cancer .The cancer-specific QOL is related to all stages of the disease. In fact, for all types of cancer patients, general QOL instruments can be used to assess the overall effect of patients' health status on their QOL, however hand cancer-specific instruments assess the effect of a specific cancer on QOL. In some cancer diseases, QOL has become an important endpoint for treatments comparison in randomized controlled trials so that in these patients clinical studies increasingly incorporate QOL as the endpoint. (**Mauer, Bottomley & Martin, 2008**).

Cancer is a devastating disease that can negatively affect a patient's QOL. Treatment for malignancies in particular, frequently involves complex, intense, and prolonged chemotherapy regimens (**Padilla & Ropka, 2005).** A chemotherapy regimen is considered to be complex and prolonged when it includes many different

agents given alone and/or in combination repeatedly over a prolonged period time. These regimens are intense because dosage reduction is seldom considered. The primary treatment outcome is to eradicate and maintain the eradication of all abnormal cells in the bone marrow (**Black, & Hawks, 2009**).

Chemotherapeutic drugs are effective destroying or preventing the application, the multiplication of cancer cells and normal tissue is also affected. The most affected cell is gastrointestinal tract, hair follicles and bone marrow. As a result, side effects can be expected from the administration of these drugs. The severity of side effects is usually related to the strength of the dose (**Cohen, De-Moor, Eisenberg, Ming& Hu, 2007**).

Side effects are classified as immediate, early, delayed, and late. An immediate side effects, early one such as hypersensitivity reaction, occurs within the first 24 hours. Diarrhea and alopecia have an onset of days to weeks and are considered early side effects. Delayed effects, such as anemia or pulmonary fibrosis occur within weeks to months, and late effects, such as second malignancies may not appear for months or years. (**Potter & Perry, 2009).**

Information about quality of life is important for planning treatment, decision making, and the provision of supportive care. Such information is useful for both clinicians and patients, as well as to promote communication between them. Oncology nurses play a critical role in providing this information to patients, so that they can evaluate the balance of risks and benefits associated with treatment **(American Cancer Society, 2010).**

One of the factors that emerge as important for the quality of life of patients with cancer is education**.** Patient education has demonstrated its potential to improve quality of life, ensure continuity of care, effectively reduce the incidence of complications of illness, promote adherence to health care treatment plans, decrease patient anxiety and maximize independence in the performance of activities of daily living. In addition, it energizes and empowers patients to become involved in the planning of teaching sessions which increases patient satisfaction **(Lewis, Heitkemper, & Dirksen, 2004**).

Significance of the study

Several studies reported that patients with cancer on chemotherapy are practicing poor health behaviors as result of severity of side effects, versus inability to manage side effects; previous researches emphasized the need for patient education, and clarification of instructions to each individual patient *(Mohamed, 2001; Mohamed, 2006).* The ability of patient with cancer to control chemotherapy side effects and maintain reasonable quality of life is limited due to lack of knowledge, guidance, and instructions from health care provider, who usually refrains from transferring responsibility for the treatment to the patient. So the education is important or needed for such group of patients treated by chemotherapy, instructions, and guidance are needed to enhance their self care abilities to overcome the problems and enhance their quality of life.

Aim of the Work

This study was aiming to assess the effect of educational intervention on quality of life for patients with cancer on chemotherapy.

Research Hypotheses

It was hypothesized that the patients who receive education will have adequate level of knowledge after one month and better quality of life scores after 6 months (physical**,** psychological, social, and spiritual) and reduction in the severity of side effects related to chemotherapy.

Subjects and Methods:

Research Design:

A quasi-experimental design was utilized in this study.

Setting:

The research was conducted in the Radiotherapy and Nuclear Medicine Center affiliated to Ain-Shams University Hospitals.

Subjects:

A purposive sample of fifty patients was consecutively recruited into this study. The sample was calculated by power and sample size calculation program to give power of 80%. The inclusion criteria were adult patients, their age ranged between 20-50 years, from both sexes and on chemotherapy; received at least 2-3 sessions of chemotherapeutic agent. Exclusion criterion was the presence of any diagnosed chronic disease such as, diabetes mellitus and hypertension.

Tools of Data Collection:

After reviewing literature, tools to be used were developed and data were collected by the researcher using the following:

1. Interviewing Questionnaire Sheet:

Constructed by the researcher, it consists of two parts:

Part 1: Patients' characteristics: They include age, sex, marital status& level of education, etc.

Part 2: Questionnaire sheet: To assess patient's level of knowledge about cancer, causes, chemotherapy, side effects, care to overcome these side effects, and relaxation techniques to reduce the side effects.

Scoring system:

Two grades were given when the response was completely correct, one grade was given when the response was incomplete, and zero was given when the response was unknown or incorrect.

2- Quality of Life Index Scale:

The scale was adopted from **Ferrell and Grant (2003**) to assess the effect of cancer on the quality of life for patients with cancer on chemotherapy. This scale was modified by the researcher to be suitable for patients with cancer on chemotherapy under the study. The quality of life scale covered four health's dimensions as the following: Physical well- being (10 items), psychological well-being (14 items), and social well-being (9 items), and spiritual well-being (7 items).

Scoring system:

According to patient's response ,the following classification was adopted normal condition(zero),mild disturbance (1), moderate disturbance (2), and sever disturbance (3).Patient's grades were collected and recorded as follows: Physical well-being (0-30), psychological well-being (0- 42), social well- being (0- 27), and spiritual well-being (0- 21) .So, the total score ranged from 0-120: Then, the total grade was evaluated as follows: poor when the total score was less than 60%, average when the total score was 60% to less than 75% and good when total score was 75% to 100%.

Validity:-

It was established for face and content by seven experts, three of them were doctors of medical staff (surgery & oncology) in El-Demerdash Hospital and the other four members from medical-surgical nursing staff at Ain Shams University (professors & assistant professors), who reviewed the tools for clarity, relevance, comprehensiveness, understanding, applicability and ease for implementation. According to their opinions minor modifications were applied.

Reliability:

Testing the proposed tool was done using Alpha Cronbach test. Results of Alpha Cronbach test was for the quality of life domains were as follows: Physical =0.927, psychological = 0.834, social = 0.959 and spiritual =0.739, indicating high reliability of quality of life scale (high internal consistency).

Proposed educational intervention:

This educational intervention was designed to improve patients' level of knowledge and their quality of life to help them understand the potential side-effects of the drugs they are receiving and learn helpful management techniques that will ensure an effective and manageable (less toxic) chemotherapy experience. It was designed in a simplified illustrated and comprehensive Arabic handout. It consists of two major sections, the first one concerned with providing the patient with the essential information about cancer, causes, treatment, administration of chemotherapy, chemotherapy side effects, nutritional manipulation, and the self care measures that patient can apply to overcome those side effects. This information was provided to the patients, in the form of handout and in addition to oral explanation. This handout describes side effects of chemotherapy on different body systems, and strategies for minimizing these side effects. The second section was concerned with teaching the patient progressive muscle relaxation technique through demonstration and redemonstration in front of patient in addition to answering patient's questions and giving feedback.

Pilot Study:

A pilot trial was carried out on five patients with the same inclusion and exclusion criteria. Based on the pilot trial findings, necessary revisions were made in the data collection forms. These five patients were not included in the main study sample.

Administrative and ethical aspects

To carry out the study in the predetermined hospitals, letters including the aim of the study were directed from the researchers' faculty of nursing to the hospital's director and also to nursing director to obtain their permission and help to conduct the study in their facility, then the researcher met them and explained the purpose of the study and methods of data collection. The researcher also obtained the study subjects' approval orally after explaining the purpose of the study and method of data collection. Confidentiality of subjects' responses was assured to them and they were informed about their right to withdraw from the study at any time without giving any reason.

Procedures

First phase:

At the beginning of this study, all patients were interviewed and asked to fill in the questionnaire sheet and quality of life index scale with the help of the researcher in the previous mentioned setting, in the same day of chemotherapy dose to assess their knowledge and quality of life. The whole field work lasted for about ten months from beginning of June 2009 to end of June 2010.

The educational intervention covered the various essential aspects related to cancer and chemotherapy and according to patient's needs. Patients were taught individually or in small groups. This intervention took a period ranging from 5-6 consecutive sessions for

patients on chemotherapy .Each session took 30-45 minutes on individual basis as it was not possible for patients be to gathered in the same sessions because they didn't come regularly to the unit and because each patient was treated as a unique case based on his needs, condition and personal characteristics.

At the beginning of the first session, patients were oriented about the content and its effects on their condition. Patients were informed about the time of the next session at the end of each session. Each session started by
a summary about what has been discussed in the previous one then the objectives of the new session were mentioned using simple arabic language. As well, the session ended by a summary of its contents and feedback from the patients was obtained to ensure that he/she got the maximum benefit.

Second phase:

Effect of the patient education on patient's condition was evaluated through comparing the pre, post one month and after six months, assessment of the patients delt with their level of knowledge and quality of life index scale. This phase was applied two times; immediate evaluation which has been done immediately post completion of the implementation phase, then post six months, the second evaluation was done.

Statistical design:

Data entry and analysis were done using the SPSS program, version 11.0 applying appropriate statistical methods i.e. descriptive statistics, Paired T- test,
ANOVA test (F- test) and student-t-test.

3. Results:

The demographic characteristics of patients involved in the study revealed that less than three fifths of patients (58%) their age ranged between 18-<45 years, 56% were females, and 64% were married . As regards level of education, 40% of patients were illiterates; half of them (50%) were working some time. Concerning monthly income, majority of them (80%) had income ranged between 300 -< 700 L.E (Table 1).

There were highly statistically significant differences in patient's level of knowledge pre/ post one month and post six months from the educational intervention about cancer in relation to all items (definition & causes of cancer, treatment of cancer, definition and purpose of chemotherapy, etc) (t1=26.36 at P<0.001). Meanwhile, there were highly statistically significant differences in patient's level of knowledge post one month and after six months in relation to all items (t2=15.9 at P<0.001) and a statistically significant difference for side effect of chemotherapy on reproductive system(t2=3 at P<0.05)(Table 2).

There were highly statistically significant differences in all items of physical health dimension of quality of life between pre and post one month of the educational intervention except for items of diarrhea/constipation and skin changes (t1= 0.25 & 1.7 respectively at P>0.05**)** **(**Table 3).As well, there were highly statistically significant differences in all physical health dimension of quality of life between pre and post six months of the educational intervention except for item of skin changes (t2=0.37 at P=>0.05) (Table 3).

There were highly statistically significant differences in all items of psychological health dimension of quality of life between pre and post one month of the educational intervention except in items of good over all quality of life, satisfying your life, ability to concentrate or remember things, and fearful from side effects of chemotherapy (t1=0.000, 0.22, 1.81 &1.85 respectively at P>0.05**)**. As well, there were highly statistically significant differences in all items of psychological health dimension of quality of life between pre and post six months of the educational intervention except in fearful from side effects of chemotherapy and fearful from spreading (t2=1.88 & 1.5 respectively at P>0.05) (Table 4).

There were a highly statistically significant differences in all items of social health dimension of quality of life between pre and post one month of the educational intervention except in support received from others and rate overall social status (t1=1.09 & 1 respectively at P>0.05). As well, there were a highly statistically significant differences in all items of social health dimension of quality of life between pre and post six month of the educational intervention except in support received from others(t2=1.46 at P>0.05) (Table 5).

There were no statistically significant differences in all items of spiritual health dimension of quality of life between pre and post one month of the educational intervention except in amount of uncertainty about future, it was highly significant (t1=3.38 at p<0.001). Meanwhile, there were a highly statistically significant differences in all items of spiritual health dimension of quality of life between pre and post six months of the educational intervention except in amount of support from religious activities, amount of support from personal spiritual activities and positive change in life due to illness (t2=1.83, 0.000 & 1.16 respectively, at P>0.05) (Table 6).

A comparison of means of patient's quality of life dimensions at pre, post one month and post six months from the educational intervention is shown in Table (7). It can be noticed that, there are highly statistically significant differences between them (77.78 ±6.54; 63.54 ±7.85; and 50.24±5.47 respectively at p<0.001).

Relations between total mean knowledge scores among patients in the study and their sociodemographic

characteristics pre, post one month and post six months from the educational intervention are displayed in table (8).There was a statistically significant difference regarding to age pre and post one month from the educational intervention and sex post six months(F2=2.02 & F3= 2.73 respectively at P<0.05).AS well, There are highly statistically significant differences regarding level of education and total knowledge scores pre, post one month and post six months from the educational intervention (F=13.73, 3.79 & 8.84 respectively at P<0.001). However, There was no statistically significant difference between patient's income and total knowledge scores pre, post one month and post six months from the educational intervention (T=1.31, 0.01& 0.11 respectively, at P>0.05).

Relations of total quality of life scores among patients in the study and their sociodemographic characteristics pre, post one month and post six months from the educational intervention; There were statistically significant differences regarding marital status pre educational intervention, level of education post one month and post six months, and patient's income post six months (F=2.3, 2.34 & 2.71; and T=2.43 respectively at P<0.05). Meanwhile, there were no statistically significant differences between patient's age and sex with total quality of life scores pre, post one month and post six months (F=0.917, 0.027, & 0.14;and T=0.317,0.804; and 0.552 respectively, at P>0.05) (Table 9).

There is obvious decrease in frequency of patient's health problems related to chemotherapy administration as reported by the studied patients pre and post six months from the educational intervention regarding to the majority of the problems as nausea, vomiting, constipation/diarrhea, altered in taste, anorexia, fatigue, anxiety and alopecia (table10).

Table (1): Sociodemographic characteristics of the study sample (n=50).

Variables	No	%
Age (years)		
18-<45	29	58
45-<60	15	30
60+	6	12
Gender		
Male	22	44
Female	28	56
Marital status		
- Single	8	16
- Married	32	64
- Divorced	5	10
- Widowed	5	10
Level of education		
- Illiterate	20	40
- Read & write/ primary	13	26
- Secondary	8	16
- University	9	18
Job status after disease		
- Working full time	10	20
- Working some time	25	50
- Leaving work	15	30
Monthly income (L.E)		
<300	6	12
300-	20	40
500-	20	40
700-900	4	8

Table (2): Comparison of patient's level of knowledge scores at pre, post one month & post 6 months of the educational intervention

Items	Mean ± SD			t1	p	t2	P
	Pre	Post one month	Post 6 months				
Definition & causes of cancer	2.7 ±1.3	5.7 ±0.7	4.7 ±1.1	16.9	<0.001**	6.9	<0.001**
Treatment of cancer	1.6 ±0.8	2.9 ±0.3	2.5 ±0.5	11.3	<0.001**	4.9	<0.001**
Definition of chemotherapy	1.4 ±0.6	2.8 ±0.4	2.3 ±0.5	12.9	<0.001**	6.2	<0.001**
Purpose of chemotherapy	1.3 ±0.5	2.7 ±0.5	2.4 ±0.6	15.5	<0.001**	3.7	<0.001**
Methods of administration	1.3 ±0.5	2.9 ±0.4	2.5 ±0.5	19.8	<0.001**	3.6	<0.001**
Side effects on integumentary system & care	4.8 ±1.7	11.1 ±1.1	9.8 ±1.8	26.5	<0.001**	6.2	<0.001**
Side effects on GIT and care	8.5 ±3.6	19.6 ±1.8	16.9 ±3.1	22.2	<0.001**	7.4	<0.001**
Side effects on neuromuscular	1.2 ±0.5	2.7 ±0.4	2.4 ±0.5	16.3	<0.001**	3.9	<0.001**
Side effects on hematopoitic	7 ±2.7	16.7 ±1.7	15.1 ±2.4	23.6	<0.001**	4.4	<0.001**
Side effects on reproductivty	2.3 ±1	5.8 ±0.5	5.4 ±0.9	20.9	<0.001**	3	<0.05*
Relaxation technique exercise	3.4 ±1.4	8.3 ±1.1	7.4 ±1.7	21.6	<0.001**	4.5	<0.001**
Total Knowledge	34.2 ±12.17	81.2 ±6.51	71.4 ±11.15	26.36	<0.001**	15.9	<0.001**

t1 (Pre Vs post one month) t2 (post one month Vs post 6 months) (*) Significant at p<0.05 (**) Highly significant at p<0.001

Table (3): Comparison of patient's physical health dimension of quality of life scores at pre, post one month & post 6 months of the educational intervention

Physical Items	Mean ± SD			t1	p	t2	P
	Pre	Post One month	Post 6 months				
1- Feeling of fatigue	2.4 ±0.8	1.8 ±0.6	0.9 ±0.5	5.2	<0.001**	12.85	<0.001**
2-Loss of appetite	2.3 ±0.8	1.7 ±0.6	0.7 ±0.5	4.95	<0.001**	12.04	<0.001**
3- Presence of pain	1.9 ±0.1	1.2 ±0.7	0.2 ±0.4	4.76	<0.001**	12.82	<0.001**
4-Sleeping disturbance	2 ±0.9	1.1 ±0.8	0.2 ±0.4	7.37	<0.001**	15.44	<0.001**
5-Nausea & vomiting	2 ±0.8	1.3 ±0.6	0.2 ±0.4	5.58	<0.001**	15.28	<0.001**
6-Diarrhea /constipation	1.2 ±1	1.2 ±0.6	0.4 ±0.5	.25	>0.05	5.02	<0.001**
7-Skin changes	0.9 ±1.1	1.2 ±0.7	0.8 ±0.4	1.7	>0.05	.37	>0.05
8-Alopecia	1.7 ±1.2	1.4 ±0.7	0.5 ±0.5	1.99	<0.05*	7.57	<0.001**
9-Dry mouth	1.2 ±1	0.7 ±0.8	0.1 ±0.3	3.18	<0.001**	7.91	<0.001**
10-Rate your physical status	1.1 ±0.8	1.1 ±0.6	2.2 ±0.6	0.16	>0.05	8.06	<0.001**

t1 (Pre Vs post one month) t2 (Pre Vs post 6 months)
( ) In significant at p >0.05 (*) Significant at p<0.05 (**)Highly significant at p<0.01

Table 4): Comparison of patient's psychological health dimension of quality of life scores at pre, post one month & post 6 months of the educational intervention

Psychological Items	Mean ± SD			t1	p	t2	P
	Pre	Post one month	Post 6 month				
1- How difficult tocope with chemotherapy?	2.32 ±0.7	1.76 ±04	0.9 ±0.6	5.62	<0.001**	14.92	<0.001**
2-Good over all quality of life	1.44 ±0.8	1.44 ±0.6	2.24 ±0.6	0.000	>0.05	6.6	<0.001**
3- Satisfying your life	1.56 ±0.8	1.58 ±0.7	2.38 ±0.6	0.22	>0.05	7.02	<0.001**
4-Ability to concentrate or remember things	2.00 ±0.7	1.78 ±0.5	2.42 ±0.6	1.81	>0.05	3.61	<0.001**
5-How useful do you feel?	2.26 ±0.8	1.76 ±0.6	1.8 ±1	3.63	<0.001**	2.1	<0.05*
6-Distressing about initial chemotherapy	2.52 ±0.6	1.66 ±0.6	0.82 ±0.6	7.77	<0.001**	12.91	<0.001**
7-Distressing about the time of chemotherapy	2.32 ±0.8	1.5 ±0.6	0.58 ±0.6	6.31	<0.001**	11.55	<0.001**
8-Feeling of anxiety	2.46 ±0.7	1.42 ±0.7	0.42 ±0.6	7.6	<0.001**	12.98	<0.001**
9-feeling of depression	2.1 ±0.9	1.42 ±0.8	0.34 ±0.6	4.81	<0.001**	12.43	<0.001**
10-Fearful from second cancer	2.6 ±0.8	2 ±0.6	1.28 ±1	4.58	<0.001**	7.65	<0.001**
11-Fearful from side effects of chemotherapy	2.7 ±0.8	2.46 ±0.8	2.4 ±0.8	1.85	>0.05	1.88	>0.05
12-fearful from spreading	2.72 ±0.8	2.46 ±0.6	2.5 ±0.7	2.16	<0.05*	1.5	>0.05
13-Rate over all psychological well being.	0.72 ±0.9	1.12 ±0.7	1.86 ±0.7	3.06	<0.05*	7.98	<0.001**

t1 (Pre Vs post one month) t2 (Pre Vs post 6months)
( ) In significant at p >0.05 (*) Significant at p<0.05 (**) Highly significant at p<0.001

Table (5): Comparison of patient's social health dimension of quality of life scores at pre, post one month & post 6 months of the educational intervention

Social Items	Mean ± SD			t1	p	t2	P
	Pre	Post one month	Post 6 months				
1-Your illness is distressing your family	2.74 ±0.5	2.6 ±0.5	1.18 ±0.9	6.73	<0.001*	11.11	<0.001*
2-Support received from others	2.62 ±0.6	2.52 ±0.6	2.42 ±0.9	1.09	>0.05	1.46	>0.05
3-To what degree a chemotherapy interfered with your personal relationship?	1.62 ±0.9	1.2 ±0.6	0.34 ±0.5	2.88	<0.05*	9.77	<0.001*
4- To what degree chemotherapy interfered with your sexuality?	1.9 ±1.2	1.08 ±0.9	0.4 ±0.7	5.56	<0.001*	9.11	<0.001*
5- To what degree chemotherapy interfered with your employment?	1.59 ±1.3	0.84 ±1.1	0.36 ±0.7	4.38	<0.001*	7.54	<0.001*
6- To what degree chemotherapy interfered with your activities at home?	2.08 ±0.7	1.58 ±0.7	0.34 ±0.6	3.71	<0.001*	14.85	<0.001*
7- Isolation caused by chemotherapy	1.66 ±0.9	1.04 ±0.5	0.22 ±0.5	4.64	<0.001*	11.51	<0.001*
8-Financial burden	1.78 ±1.1	1.22 ±0.6	0.46 ±0.6	3.91	<0.001*	7.87	<0.001*
9-Rate overall social status	1.04 ±0.6	1.16 ±0.6	2.22 ±0.8	1	>0.05	8.87	<0.001*

t1 (Pre Vs post one month) t2 (Pre Vs post 6months)
( ) In significant at p >0.05 (*) Significant at p<0.05 (**) Highly significant at p<0.001

Table (6): Comparison of patient's spiritual health dimension of quality of life scores at pre, post one month & post 6 months of the educational intervention

Spiritual Items	Mean ± SD			t1	p	t2	P
	Pre	Post one month	Post 6 month				
1-Amount of support from religious activities	2.48 ±0.6	2.58 ±0.5	2.64 ±0.5	1.04	>0.05	1.83	>0.05
2-Amount of support from personal spiritual activities	2.48 ±0.6	2.58 ±0.5	2.58 ±0.6	1.15	>0.05	0.00	>0.05
3-Amount of uncertainty about future	2.28 ±1	1.32 ±0.9	0.32 ±0.6	3.38	<0.001*	12.75	<0.001**
4-Postive change in life due to illness	0.24 ±0.7	0.28 ±0.8	0.4 ±0.8	0.33	>0.05	1.16	>0.05
5-Sense of being a life	2.4 ±0.9	2.46 ±0.6	2.7 ±0.5	0.44	>0.05	2.28	<0.05*
6-Do you feel hopeful	1.7 ±0.9	1.72 ±0.7	2.24 ±0.7	0.14	>0.05	3.69	<0.01*
7-Rate over all spiritual well being	1.36 ±0.6	1.48 ±0.6	0.32 ±0.6	1.06	>0.05	8.99	<0.001**

t1 (Pre Vs post one month) t2 (Pre Vs post 6months)( ) Insignificant at p >0.05 (*) Significant at p<0.05 (**)Highly significant at p<0.001

Table (7): Comparison of total means of patient's quality of life dimensions at pre, post one month and post six months from the educational intervention

Items	Mean ± SD	t- test	P-value
Total quality of life dimensions pre educational intervention.	77.78 ± 6.54	Pre & post 12.25	<0.001**
Total quality of life dimensions post one month educational intervention.	63.54 ± 7.85	Pre & follow up 30.1	<0.001**
Total quality of life dimensions post six months from educational intervention (follow up).	50.24 ± 5.47	Post& follow up 20.01	<0.001**

(**)Highly significant at p<0.01

Table (8): Relations between total mean knowledge scores among patients under study and their sociodemographic characteristics

Socio -demo graphic characteristics	Total Knowledge Scores						F- test	P-value
	Pre		Post one month		Post six months			
	Mean	±SD	Mean	±SD	Mean	±SD		
Age(years)								
18-< 45	33.61	11.65	82.33	5.62	72.61	10.4	***F1*** 0.374	***p1*** >0.05
45- <60	34.15	13.48	79.75	6.4	70.77	11.31	***F2*** *2.02*	***P2*** <0.05*
60+	39.25	14.36	76.25	11.73	64	16.55	***F3*** 1.1	***P3*** >0.05
Gender							**Student t- test**	
Male	33.68	10.03	83.21	5.43	76.53	9.95	***t1*** 0.232	***p1*** >0.05
Female	34.52	13.47	79.97	6.89	68.32	10.82	***t2*** 1.84	***P2*** >0.05
							t3 2.73	***P3*** <0.05*
Marital status								
Single	38.43	11.09	87	0	84.29	5.62	***F1*** 2.16	***P1*** <0.05*
Married	31.86	10.92	81.16	5.81	70.43	9.7	***F1*** 4.76	***P2*** <0.05*
Divorced	41.33	16.65	75	1	59.33	2.52	***F2*** 6.05	***P3*** <0.001**
Widowed	46	19.67	74.33	13.58	66	19.67		
Level of education								
Illiterate	28.86	2.27	78.73	5.74	66.32	9.08	***F1*** 13.73	***P1*** <0.001**
Read & write	30.5	4.4	83.17	5.24	73.42	8.88	***F2*** 3.79	***P2*** <0.01**
Secondary	37.33	14.01	80.11	9.06	69.67	12.49	***F3*** 8.84	***P3*** <0.001**
University	53.29	17.97	87	0	86.43	1.51		
Income							**Student t- test**	
Enough	35.38	14.17	81.21	6.75	71.32	11.73	***t1*** 1.31	***P1*** >0.05
Not enough	31.69	5.65	81.19	6.2	71.69	10.14	***t2*** 0.01	***P2*** >0.05
							t3 0.11	***P3*** >0.05

( ) In significant at p >0.05; F1, t1& P1 for pre;
(*) Significant at p <0.05; F2, t2 & p2 for post one month (**) Highly significant at p<0.001; F3, t3 & p3 for post six months

Table (9): Relations between total quality of life mean scores among patients under study and their sociodemographic characteristics

Sociodemographic characteristics	Total Quality of Life Scores						F - test	P-value
	Pre		Post one month		Post six months			
	Mean	±SD	Mean	±SD	Mean	±SD		
Age(years)								
18-< 45	78.55	5.8	63.73	7.85	49.94	5.84	***F1*** 0.917	***P1 >0.05***
45-< 60	76.72	8.59	63.15	8.9	50.85	5.13	***F2*** 0.027	***P2*** >0.05
60+	0.74	4.44	63.25	5.74	50.75	4.11	***F3*** 0.14	***P3 >0.05***
Gender							**Student t- test**	
Male	78.16	5.29	64.68	7.78	50.79	3.84	***t1*** 0.317	***P1 >0.05***
Female	77.55	7.28	62.84	7.94	49.9	6.3	***t2*** 0.804	***P2*** >0.05
							t3 0.552	***P3 >0.05***
Marital status								
Single	74.29	5.5	62.29	5.79	49.71	2.43	***F1*** 2.3	***P1*** <0.05*
Married	79.05	5.96	63.95	8.24	50.57	6	***F2*** 0.26	***P2 >0.05***
Divorced	71.33	10.79	60.33	8.96	45.33	3.22	***F3*** 1.019	***P3 >0.05***
Level of education								
Illiterate	77.68	7.05	60.86	7.65	48.41	4.09	***F1*** 0.173	***P1 >0.05***
Read & write	77.33	5.96	64.25	8.54	52.33	5.61	***F2*** 2.34	***P2*** <0.05*
Secondary	77.33	5.98	65.44	7.55	49.22	3.73	***F3*** 2.71	***P3*** <0.05*
University	79.43	7.66	68.29	5.49	53.71	8.52		
Income							**Student t- test**	
Enough	78.26	6.41	63.35	7.4	50.18	5.79	***t1*** 0.761	***P1 >0.05***
Not enough	76.75	6.9	63.94	8.9	50.38	4.88	***t2*** 2.43	***P2*** <0.05*
							t3 0.12	***P3 >0.05***

( ) In significant at p >0.05
(*) Significant at p <0.05
(**)Highly significant at p<0.001

F1, t1& P1 for pre
F2, t2 & p2 for post one month
F3, t3 & p3 for post six months

Table (10): Frequency of health problems related to chemotherapy administration as reported by the studied patients at pre; post one month and post six months from the educational intervention.

variables	Pre		Post one month		Post six months	
	No	%	No	%	No	%
Nausea	48	96	47	94	13	26
Vomiting	40	80	2	4	0	0
Mucositis	11	22	8	16	4	8
Constipation / diarrhea	41	82	46	94	22	44
Bleeding	3	6	5	10	3	6
Altered taste	47	94	37	74	22	44
Anorexia	48	96	37	74	20	40
Dry mouth	20	40	10	20	5	10
Fever	24	48	9	18	4	8
Fatigue	48	96	39	78	35	70
Anxiety	46	92	7	14	7	14
Difficult swallowing	17	34	2	4	1	2
Heart burn	12	24	3	6	2	4
Weight loss	26	52	39	78	31	62
Alopecia	44	88	33	66	4	8
Other problems	30	60	0	0	0	0

4. Discussion:

Quality of life has become an important issue in evaluating the effectiveness of health care, especially in the field of cancer care, where treatment is often debilitating (***Bondini et al., (2007)***. Now QOL assessment is considered one of the clinical standard outcomes ***(Amgen, 2006).*** Patients' education has demonstrated its potential to improve QOL, ensure continuity of care, effectively reduce the incidence of complications of illness, promote adherence to health care treatment plans, decrease patient anxiety and maximize independence in the performance of activities of daily living. In addition, it energizes and empowers patient to become involved in the planning of teaching session that increases patient satisfaction ***(Black & Hawks, 2009)***. So, the aim of this study was to assess the effect of educational intervention on quality of life for patients with cancer on chemotherapy.

As regards age, less than three fifths of the sample

is in middle adulthood that is characterized by work and being a productive person for both the family and society. So, feeling of not being able to perform social roles could affect the person's quality of life. This result comes in agreement with a study done by **Abd *El-Moneem* (2004),** who assessed quality of life among cancer patients receiving opioids which revealed that the majority of sample was in the middle adulthood. However, this result disagrees with that of a study carried out by ***Ibrahim (2001),***on self- care practices of cancer patients undergoing chemotherapy which revealed that the majority of patients were 50 years old and more.

According to the present study findings, more than half of sample were females, and the study results revealed no statistically significant difference detected between male and female patients in relation to total mean scores of quality of life. This result comes is congruent with that of a study carried out by ***Abd Elhy(2004),*** that assessed self- esteem among cancer patients receiving chemotherapy in Shebin EL Kom University, which reported that the majority of the sample (80%) were females.

In relation to educational level, illiteracy or just abilities to read and write was prevalent among almost two thirds of these study sample. Educational level had no statistically significant difference in QOL. This conclusion comes in agreement with a study done by ***Abd ELmoneem (2004),*** on Egyptians with cancer receiving opioids, which revealed that the educational level had no statistically significant difference in QOL. On the other hand, ***Chen, Wang and Zhu (2007)*** mentioned that patients with higher education had high global health status and had better QOL.As well, ***Mouw, Koster and Wright (2008)*** clarified that the higher level of educational attainment had lower cancer risk.

Concerning patient's monthly income, the present study results revealed that the majority of the studied patients had moderate income, however after the disease occurrence, almost half of them required reduced work, and about one third of them became unemployed. This means that their moderate income wasn't sufficient to meet the costs of the treatment and their family needs, so feeling of inability to perform family role would certainly have had negative impact on their quality of life. This finding was supported by ***Hanafy (2003)*** who mentioned that in reference to the employment status; thirty percent of the study sample became unemployed and delegated this responsibility to their sons.

The study result revealed that there was no statistically significant relation between patient's total mean scores of quality of life and all sociodemographic characteristics. This is in accordance with ***Hassan and Robert (2007***), and ***Dehkordi,Heydarnejad and Fatehi (2009)***, who found that there was no correlation between QOL and variables such as age, sex, marital status and economic conditions. This finding may indicated that the most vulnerable groups who showed lower quality of life were patients aged over 60 years , divorced females, and manual workers (unemployed),and those who read and write, while the least vulnerable were patients aged 18- 44 years old, married and with university education.

As regards knowledge assessment, there were statistically significant improvements in all items of knowledge scores for chemotherapeutic patients post educational intervention compared with pre intervention. These results were in agreement with ***Guren, Dueland and Skovlund (2003)***, who stated that knowledge of how patients perceive the treatment-related toxicity is important in order to prepare them for what to expect during and after treatment. This was attributed to the positive effect and importance of patient's education of different modalities in managing the disease and side effects of chemotherapy to improve QOL and this finding proved this study hypothesis. However, post six months, there were inadequacies in total knowledge scores than post educational intervention. This result could be due to forgetting some factors and moving the patients to another type of treatment such as radiotherapy.

The present study finding also revealed that, there were no statistically significant relations between patient's knowledge and others sociodemographic characteristics such as age, marital status, level of education and income. This result disagreed with ***Ali (2004***), who stated that the subject's information level was statistically significant and negatively correlated with the age, while a positive association was detected between educational level and subject's knowledge level.

Post educational intervention, results of present study indicated that there was slight reduction in post chemotherapy nausea and vomiting as shown by the difference between means, however, after six months of educational intervention there was a highly statistically significant reduction in severity of post-chemotherapy nausea and vomiting after exposure to the educational intervention, and teaching the patient the relaxation technique as an effective non-pharmacological intervention for anticipatory nausea and vomiting, which developed during the course of chemotherapy. These findings were supported by the previous reports of ***Molassiotis (2000) and Ali (2004*).** The results of their studies suggested that progressive muscle relaxation training was effective in reducing the duration and intensity of post-chemotherapy nausea and vomiting.

Conclusion:

Educational intervention showed a significant effect as there was remarkable increase in patient's level of knowledge related to chemotherapy, its purpose, side effects and how to overcome chemotherapy side effects. As regards quality of life of patients, by comparing the results in the pre- and post-educational intervention, the results showed a slight improvement in patient's quality of life post one month, while post six months there was general improvement in all dimensions of quality of life; physical, psychological, social, and spiritual status.

Recommendations:

Based on the results of the current research, the following suggestions for future research and practice are proposed**:**

1- Cancer rehabilitation program for patient treated by chemotherapeutic agent must be implemented through the collaboration of various rehabilitation team members.

2- Hospital teaching methods must be examined so that patient teaching is individualized and available at time when the patient is ready to learn. New methods of teaching are to be applied using new technologies such as computer assisted instructions and home videos.

3- Further investigation could be undertaken to document the cost effectiveness of educational effects in reducing hospital stay, decreasing readmission, improving the patient's quality of life and minimizing complications of illness and therapies.

4- Replication of the current study on a larger probability sample is recommended to achieve generalization and wider utilization of the designed educational intervention.

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Characterization of Phospholipase C Productivity by *Pseudomonas aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus* isolates

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Abstract: Of 205 bacterial isolates, 28 have shown high phospholipase production. Three isolates, identified as *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Staphylococcus aureus*, have shown the highest PLC productivity compared to isolates from the same morphological class and were selected for characterization of enzyme production. PLC production by these isolates took place in phosphate starved media (PS-TMM). For *Pseudomonas aeruginosa* D183, PS- TMM supported maximum PLC production. Replacing glucose with other carbon sources at different concentrations, increasing glucose concentration, adding organic proteins (except for peptone at 0.5% and BSA at 1.5%), or replacing metal salt components of PS-TMM, inhibited PLC production. PS- TMM, to which BSA (1 or 1.5%) or sodium cholate (0.05%) was added, supported maximum PLC production by *Bacillus cereus* D101. PLC production by this isolate was increased with increasing glucose concentration in PS-TMM, while replacing glucose with other carbon sources either decreased or abolished PLC production. Adding BSA, triton X-100, sodium cholate (at 0.05 and 0.1%), tween 20, or tween 80 to PS-TMM or removing $CaCl_2$ from it increased PLC production by this isolate. Production of PLC by *Staphylococcus* isolate in PS-TMM occurred only when glucose concentration was increased to 110 mM, when BSA (0.5, 1 or 1.5%) or sodium cholate (1.25 or 2.5%) was added. PLC production by all three isolates was growth associated. Optimum pH for PLC production by the three isolates was pH 7-8 while optimum temperatures were 37˚C (*Pseudomonas aeruginosa* D183), 30˚C (*Bacillus cereus* D101) and 25-37˚C (*Staphylococcus aureus* D173).

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Keywords: Phospholipases C, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, Production, Characterization.

Abbreviations: BSA, bovine serum albumin; CF, cystic fibrosis; CFU, colony forming unit; CSF, cerebrospinal fluid; DAG, diacyl Glycerol; NPPC, p-nitrophenyphosphorylcholine; PI-PLC, phosphatidylinositol-specific phospholipase; Pi, inorganic phosphate; PL, phospholipase; PLA_1, phospholipase A_1; PLA_2, phospholipase A_2; PLB, phospholipase B; PLC, phospholipase C; PLD, phospholipase D; PS-TMM, phosphate starved tris minimal medium; Pz, phospholipase activity; TMM, tris minimal medium

1. Introduction:

Phospholipases are a heterogeneous group of enzymes that share the ability to hydrolyze one or more ester linkage in glycerophospholipids. Qualifying letters are used to differentiate among phospholipases and to indicate the specific bond targeted in the phospholipid molecule. Phospholipase A_1 (PLA_1) hydrolyzes the fatty acyl ester bond at the sn-1 position of the glycerol moiety, while phospholipase A_2 (PLA_2) removes the fatty acid at the sn-2 position of this molecule. Phospholipase C (PLC) hydrolyzes the phosphodiester bond in the phospholipid backbone, whereas the second phosphodiester bond is cleaved by phospholipase D (PLD) (Ghannoum, 2000). The diversity of roles that phospholipases play in biology and medicine is exceptional. In the past decade, this class of enzymes has proven to be considerably more complex than initially perceived and their impact on an assortment of basic cellular processes in eukaryotes, including oncogenesis and inflammation has become widely appreciated. Likewise, there are sundry functions for phospholipases in prokaryotic biology, including their noteworthy contributions to microbial virulence (Vasil, 2006). The most important class of phospholipases that have been thus far shown to play a significant role in bacterial pathogenesis is the phospholipase C (PLC) (Johansen *et al.*, 1996). In 1941, Macfarlane and Knight demonstrated that the highly cytotoxic α-toxin of *Clostridium perfringens* has PLC activity. Since then, PLC activity has been demonstrated in a variety of other pathogenic bacteria, including *Pseudomonas aeruginosa* (Ostroff and Vasil, 1987; Stonehouse *et al.*, 2002; Barker *et al.*, 2004), *Bacillus cereus* (Verkleij *et al.*, 1973; Kreft *et al.*, 1983), *Staphylococcus aureus* (Marques *et al.*, 1989), *Legionella pneumophila* (Dowling *et al.*, 1992), *Helicobacter pylori* (Drazek *et al.*, 1995), *Mycoplasma* spp. (De Silva and Quinn, 1987), *Listeria*

monocytogenes (Camilli *et al.*, 1991; Marquis *et al.*, 1997), *Mycobacterium tuberculosis* (Johansen *et al.*, 1996), and others.

This study aimed at detecting the production of phospholipases among different bacterial isolates and characterizing the productivity of phospholipases C from some clinical bacterial isolates.

2. Material and Methods

Chemicals

All chemicals were of high quality from available grades supplied (unless otherwise indicated) by El-Nasr Chemicals (Adwic), Egypt.

Bacterial Isolates and their Maintenance

Isolates D101, D173, and D183 were obtained through screening of 205 clinical isolates for phospholipase production. The isolates were recovered from 210 pathological specimens of different sources. The isolates were maintained onto nutrient agar (Difco) slants at 4ºC and subcultured every month. Glycerol stocks were used for the long term preservation of the isolates (Miller, 1972).

Growth Conditions.

1) **Primary Screening**

The solid medium used for screening the isolates was egg-yolk nutrient agar prepared by aseptically beating up one egg-yolk with 15 ml sterile saline using sterile magnet and a stirrer, then mixing with 85 ml sterile molten (60˚C) nutrient agar (Difco) and pouring in Petri dishes, 20 ml each (Collee *et al.*, 1996).

2) **Secondary Screening**

Trypticase soy broth (Difco) was used.

3) **Characterization of Enzyme Productivity**

Phosphate starved tris minimal medium was used in which the concentration of the phosphate component (KH_2PO_4) of TMM was reduced to 0.1 mM. TMM has the composition: 100 mM Tris-HCl [pH 7.2], 11 mM glucose, 5 mM NH_4Cl, 10 mM KH_2PO_4, 0.5 mM K_2SO_4, 0.1 mM $CaCl_2$, 10 mM $MgCl_2$. For Mg(II)-starved cultures, the concentration of $MgCl_2$ was reduced to 0.01 mM and the $CaCl_2$ was omitted. For phosphate-starved cultures, KH_2PO_4 was reduced to 0.1 mM. For nitrogen-starved cultures NH_4Cl was reduced to 0.5 mM (Norte *et al.*, 2003).

Inoculum Preparation

Cells from 1-day-old cultures on nutrient agar (Difco) slants were harvested in sterile normal saline. The final count in the cell suspension was adjusted to about 2×10^8 CFU/ml turbidimetrically at 640 nm with reference to calibration curves constructed for each isolate between turbidity of the bacterial suspension and the bacterial count. For secondary screening and characterization of enzyme productivity aliquots (1 ml) from the inoculums prepared above was used to inoculate 9 ml culture medium within 50 ml Erlenmeyer flasks. Incubation was done at 37˚C in a platform orbital shaker (Orbital Shaker, SO1, Stuart Scientific, UK) at 225 rpm for 48 h.

. For studying the effects of carbon sources, protein sources, and surface active agents on productivity, the phosphate starved tris minimal medium (PS-TMM) was used as a basal medium. The effect of different metal ions was studied in two steps. The first step was the effect of the separate or combined removal of the metal salt components of phosphate-starved TMM leaving only KH_2PO_4 unchanged, as well as the replacement of K_2SO_4 by Na_2SO_4, at the same concentration. The second step was the effect of addition of metal ion salts to basal phosphate-starved TMM from which all metal ion salts have been removed except for KH_2PO_4. For investigation of the effects of inorganic phosphate, incubation time, initial pH and incubation temperature on productivity, PS-TMM was used for isolate D183, and was supplemented with 1.5% BSA for isolate D173 and with 0.05% sodium cholate.

Enzyme Assays

Primary screening was done using the egg-yolk plate method where isolates were spot inoculated on the surface of egg-yolk agar plates and incubation was done 37ºC for 48-72 h. Phospholipase activity (Pz) was expressed in terms of the ratio between the diameter of the colony to the diameter of the zone of precipitation (Price *et al.*, 1982). The evaluation of phospholipase producing isolates was done as described by Dagdeviren *et al.* (2005) where a Pz of 1.0 was evaluated as negative (-), 0.99–0.9 as weak (+), 0.89–0.8 as mild (++), 0.79–0.7 as relatively strong (+++) and <0.69 as very strong (++++) activity. Isolates that showed very strong phospholipase productivity (Pz < 0.6) were further evaluated in shake-flask fermentations for their extracellular PLC production.

Phospholipase C assay was performed by the method of Kurioka and Matsuda (1976). The assay was adapted to a microtiter system by Berka and Vasil (1981). At the end of the incubation period, cells were removed from 1 ml of culture by centrifugation (Beckman Microfuge). After centrifuging, 10 µl of the clear supernatant fluid was added to 90 µl of NPPC reagent in a microtiter test plate (96 wells per plate). The NPPC reagent contained 250 mM tris(hydroxymethyl)- aminomethane-hydrochloride buffer (pH 7.2) (Oxoid), 60% glycerol (wt/wt), 1.0 µM $ZnCl_2$, and 10 mM NPPC (Sigma Chemical Co., St.

Louis, Mo.). The plates were then incubated at 37°C for 17 h before the absorbance at 405 nm was measured with MicroReader 4 plus microplate reader (Hyperion. Inc., USA). A yellow color was developed in positive cases. A blank containing 10 µl of the clear growth supernatant fluid and 90 µl of NPPC reagent that lacked the chromogenic substrate, and a control containing 10 µl of the uninoculated culture medium and 90 µl of NPPC reagent were treated similarly and included in runs of enzymatic assay.

Biomass Determination

The cell mass obtained by centrifugation of a known volume of culture broth at 12,000 rpm for 15 min was washed twice with sterile saline and resuspended in the same volume of sterile saline. Biomass was determined turbidimetrically at 640 nm in an Ultrospec 2000 spectrophotometer (Amersham Pharmacia Biotech, Piscataway, NJ, USA) with reference to calibration curves constructed for each isolate between turbidity of the bacterial suspension and the bacterial count determined using the viable count technique.

Isolate Identification

Identification was done according to Claus and Berkeley (1986), Schleifer (1986) and Palleroni (1989) in Bergey's Manual of Systemic Bacteriology.

3. Results

Screening the Collected Isolates for Phospholipase Production

Of the collected 205 bacterial isolates, 43 bacterial isolates having different morphological characters were found to produce phospholipases (Table 1). Phospholipase producing isolates were evaluated (Table 2) and isolates that showed very strong phospholipase productivity (Pz < 0.6) were further evaluated for their extracellular PLC production assay (Table 3). The isolate designated D183 gave the highest PLC productivity among the Gram-negative rods isolates, the isolate D173 gave the highest PLC productivity among the Gram–positive cocci in clusters isolates, and the isolate D101 was the highest PLC producer among the Gram-positive thick rods isolates. Therefore, these three isolates were selected for further studies and were identified as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus cereus* respectively.

Table 1. Prevalence of phospholipase production among screened bacterial isolates.

Isolate Category	Microscopical Characters	Total number of screened isolates	Number of PL producing isolates	Number of PL producing isolates in each category (% of PL producing isolates in each category)
Gram-negative	Rods	103	26	115 (22.6)
	Coccobacilli	12	0	
Gram-positive	Cocci in clusters	60	12	90 (18.9)
	Thick rods	5	5	
	Diplococci	22	0	
	Cocci in chains	2	0	
	Cocci in tetrads	1	0	
		∑ 205	**∑ 43**	

Table 2. The level of phospholipase production by the producing isolates.

Microscopical Characters	Number of isolates showing phospholipase activity of:			
	++++	+++	++	+
Gram-negative rods	14	1	9	2
Gram-positive cocci in clusters	9	2	1	0
Gram-positive thick rods	5	0	0	0
	∑ 28	∑3	∑ 10	∑ 2

++++, very strong activity (Pz <0.69); +++, relatively strong activity (Pz = 0.79–0.7); ++, mild activity (Pz = 0.89–0.8); +, weak activity (Pz = 0.99–0.9).

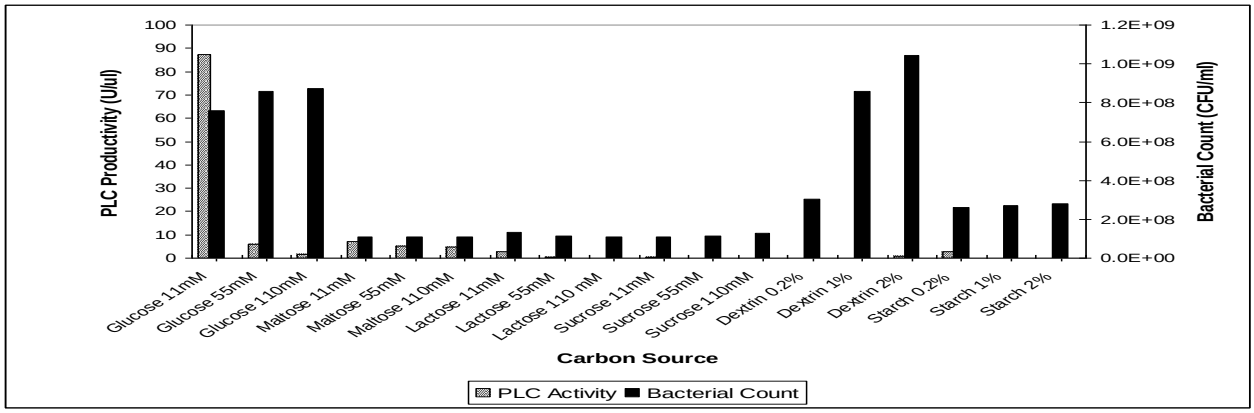
Table 3. PLC production by selected isolates in secondary screening tests.

Microscopical Character	Isolate Code	Viable Count	Production (Unit/ml)	Specific Productivity (Unit/10^6 CFU)
Gram- negative rods	D23	1.25E+10	9.72E+04	7.775
	D52	1.47E+10	8.73E+04	5.936
	D53	1.59E+10	6.64E+04	4.175
	D54	1.00E+10	9.02E+04	9.020
	D71	1.25E+10	9.90E+04	7.919
	D94	1.08E+10	5.88E+03	0.545
	D117	1.16E+10	1.22E+04	1.055
	D155	1.63E+10	4.93E+04	3.027
	D169	1.47E+10	5.23E+04	3.557
	D180	1.28E+10	1.31E+05	10.263
	D182	1.09E+10	9.23E+04	8.466
	D183	1.35E+10	1.69E+05	12.569
	D203	1.34E+10	1.70E+04	1.272
	N10	1.50E+10	4.70E+04	3.133
Gram-positive cocci in clusters	D95	3.56E+08	2.97E+03	8.344
	D119	3.43E+08	4.15E+03	12.099
	D163	3.70E+08	3.17E+03	8.567
	D166	3.75E+08	3.87E+03	10.328
	D167	3.88E+08	1.93E+03	4.966
	D172	3.29E+08	2.68E+03	8.138
	D173	3.38E+08	5.67E+03	16.776
	D193	3.07E+08	1.31E+03	4.279
	D195	3.31E+08	3.04E+03	9.174
Gram-positive thick rods	D92	3.00E+09	3.57E+04	11.892
	D93	2.17E+09	7.91E+03	3.639
	D99	2.35E+09	6.37E+03	2.712
	D101	2.48E+09	3.87E+04	15.615
	N9	2.63E+09	1.32E+04	5.002

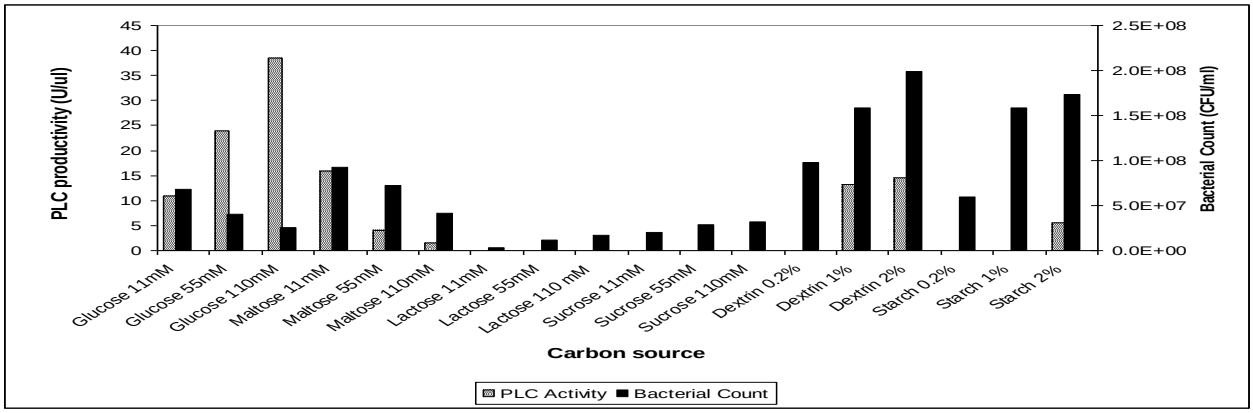
Effect of Different Carbon Sources and their Concentrations on the Production of PLC

For ***Pseudomonas aeruginosa*** **D183**. Increasing the concentration of glucose or replacing it with any of the tested carbon sources, at any of the tested concentrations, has led to a sharp decrease in PLC production. Higher growth levels were obtained with glucose (at all three tested concentrations) and with dextrin (at 1% and 2% concentrations) than any other tested carbon source (Figure 1a). However for ***Bacillus cereus*** **D101**, the increase in glucose concentration of phosphate-starved TMM was accompanied by an increase in PLC production. Yet, this wasn't the case with maltose which induced a higher level of PLC production than glucose at 11 mM concentration, but lower levels at 55 and 110 mM (Figure 1b). The replacement of glucose with lactose or sucrose promoted low levels of growth and no PLC production. Alternatively, dextrin and starch promoted high levels of growth but PLC production was detected only at the higher concentrations (1 and 2% for dextrin and 2% only for starch). For ***Staphylococcus aureus*** **D173,** poor microbial growth and PLC production were observed with all the tested carbohydrate sources, except for glucose at the higher concentrations (Figure 1c). When glucose at 55 mM was used, good growth was observed but without PLC production, while at 110 mM, good growth and PLC production were detected.

a) ***Pseudomonas aeruginosa*** **D183.**



b) ***Bacillus cereus*** **D101.**



c) ***Staphylococcus aureus*** **D173.**

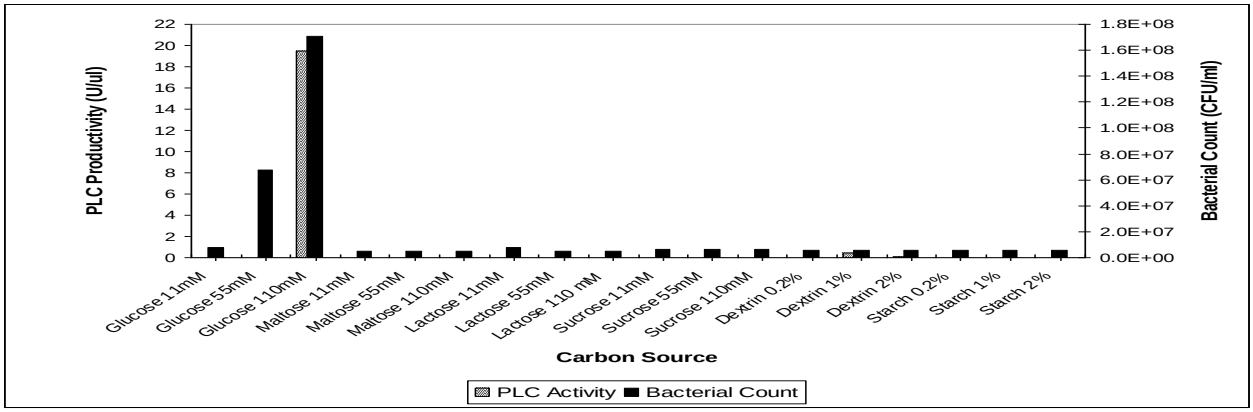


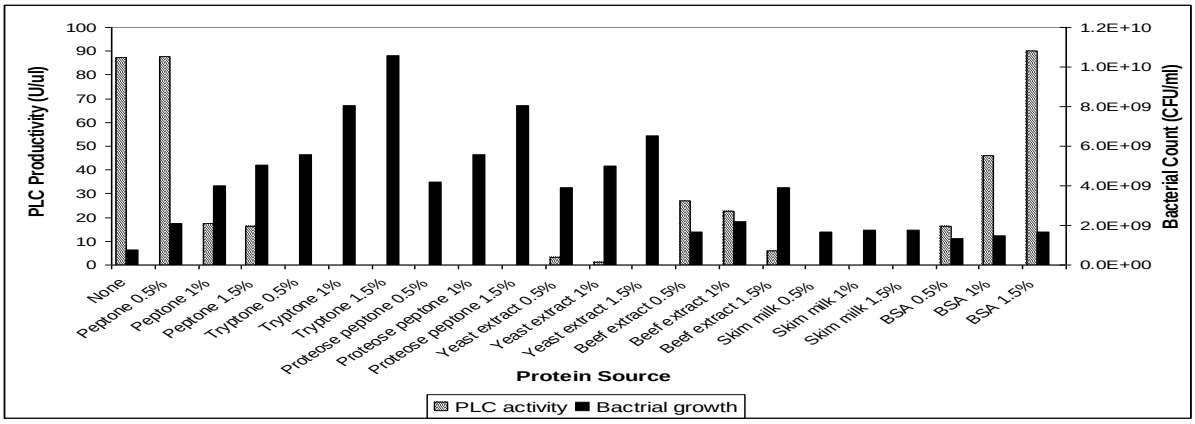
Figure 1. Effect of different carbon sources on growth and PLC production by *Pseudomonas aeruginosa* D183, *Bacillus cereus* D101 and *Staphylococcus aureus* D173.

Effect of Addition of Different Protein Sources on the Production of PLC

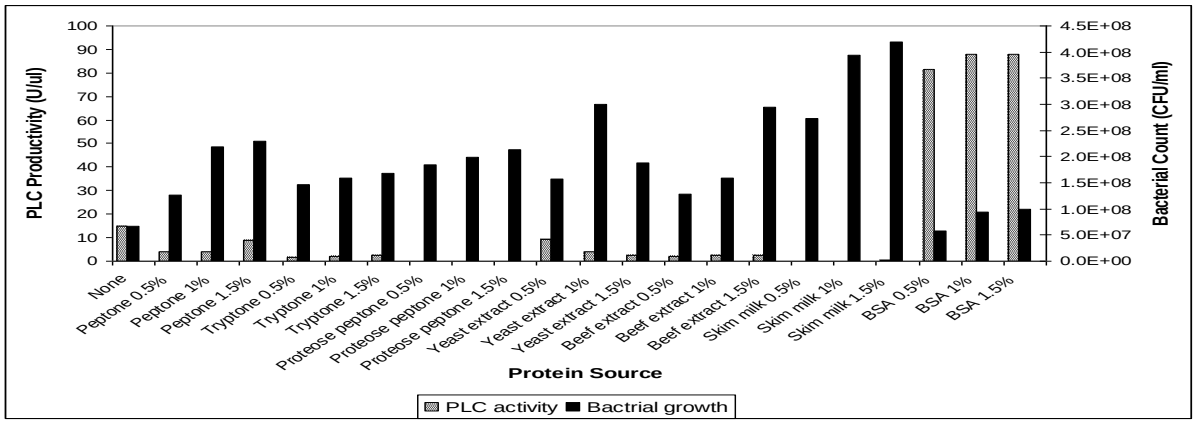
The growth of ***Pseudomonas aeruginosa*** **D183** increased upon the addition of the tested protein sources increased; however, with the exception of peptone, beef extract and bovine serum albumin, nearly no PLC production was observed by the addition of the tested protein sources at all the tested concentrations (Figure 2a). The production has fallen to zero with the addition of tryptone, proteose peptone and skim milk. The addition of beef extract resulted in low levels of PLC production and the levels decreased as the concentration of beef extract increased. Peptone at 0.5% did not affect enzyme production, but at higher concentrations, growth increased and enzyme production decreased. The increase in concentration of bovine serum albumin did not lead to a significant increase in growth; however, the inhibitory effect on PLC production has decreased until production restored its

levels as in phosphate-starved TMM at bovine serum albumin concentration 1.5%. As per ***Bacillus cereus* D101,** all the tested protein sources had a positive effect on the growth of the test isolate; however, their effects on PLC production were variable (Figure 2b). The addition of peptone, tryptone, yeast extract and beef extract has led to a decrease in PLC production. Both proteose peptone and skim milk when separately added to the phosphate-starved TMM abolished PLC production. On the other hand, the addition of bovine serum album resulted in a marked increase in PLC production (88 U/µl at 1 and 1.5% concentrations). For ***Staphylococcus aureus* D173**, although good microbial growth was obtained with all the protein sources tested, high yields of PLC were obtained only when bovine serum albumin was added to the phosphate-starved TMM (Figure 2c). Both growth and PLC production increased gradually by increasing the concentration of added bovine serum albumin.

a) ***Pseudomonas aeruginosa* D183.**



b) ***Bacillus cereus* D101.**



c) ***Staphylococcus aureus* D173**

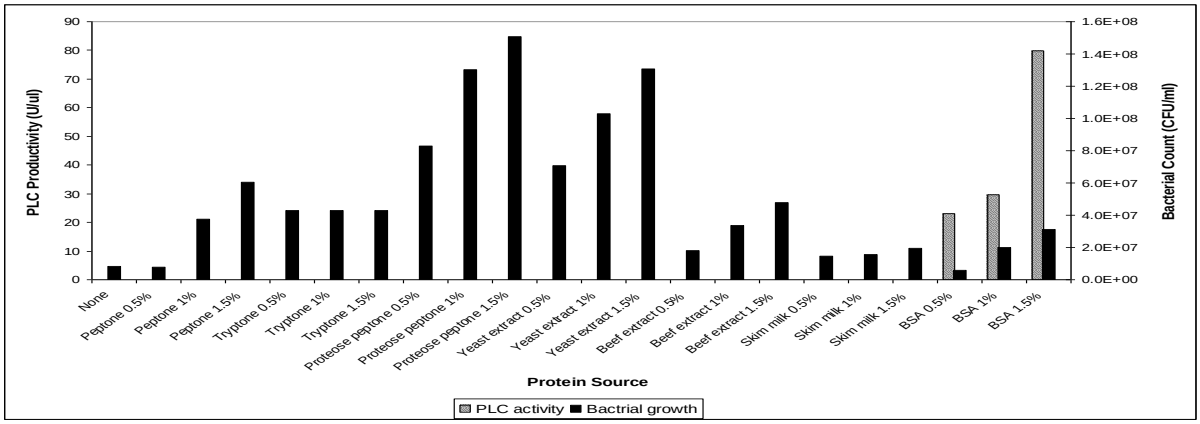
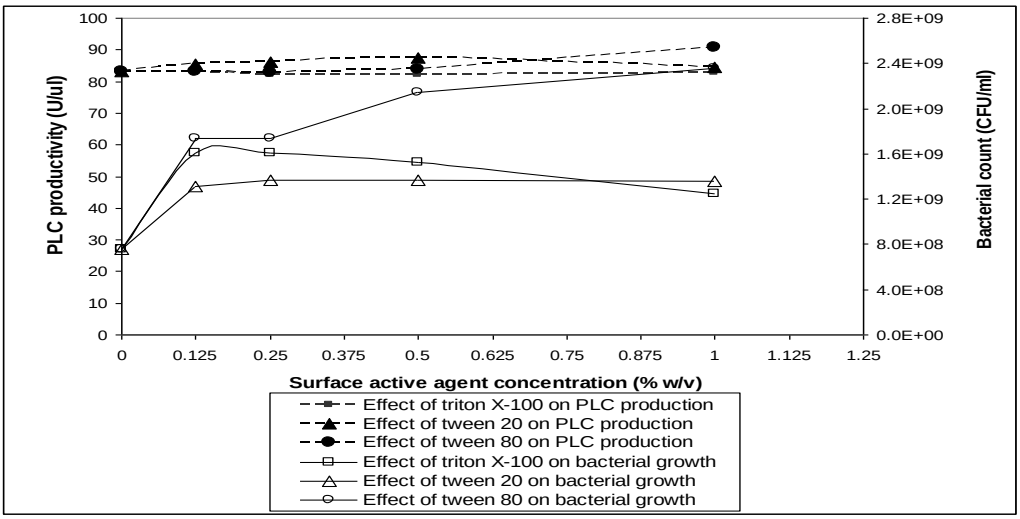


Figure 2. Effect of different protein sources on growth and PLC production by *Pseudomonas aeruginosa* D183, *Bacillus cereus* D101 and *Staphylococcus aureus* D173.

Effect of Addition of Different Surface Active Agents on the Production of PLC

The addition of triton X-100 or tween 20 to phosphate-starved TMM did not affect PLC production. However, the addition of tween 80 and sodium cholate slightly increased PLC production (less than 15% increases in both cases) by ***Pseudomonas aeruginosa*** **D183** (Figure 3). However for ***Bacillus cereus*** **D101**, the addition of triton X-100 to phosphate-starved TMM has led to a sharp decrease in growth of the test isolate, accompanied by an increase in PLC production. Tween 20 and Tween 80 have also increased PLC production by the test isolate, but the increase was accompanied by a gradual decrease in growth (Figure 4a). Sodium cholate had a positive effect on both growth and enzyme production at 0.05% (w/v) concentration, but at 0.1% (w/v) a sharp decline in growth was detected. At higher concentrations, 0.2 and 0.4% (w/v), neither growth nor enzyme production was detected (Figure 4b). As per ***Staphylococcus aureus*** **D173,** all the tested surface active agents caused a slight increase in growth of the test isolate, except for tween 20, which caused a considerable increase in growth at 0.125 and 0.25% (w/v) as compared to the other tested concentrations. While triton X-100**,** tween 80 and sodium cholate were unable to support PLC production at the tested concentrations, tween 20 supported very low levels of production at 0.125% and 0.25% (w/v) concentrations (Figure 5).

a) Effect of triton X-100, tween 20 and tween 80.



b) Effect of sodium cholate

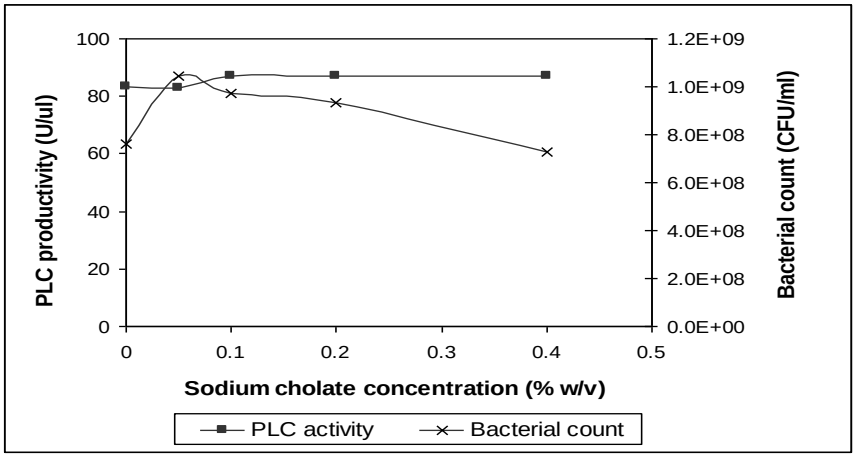
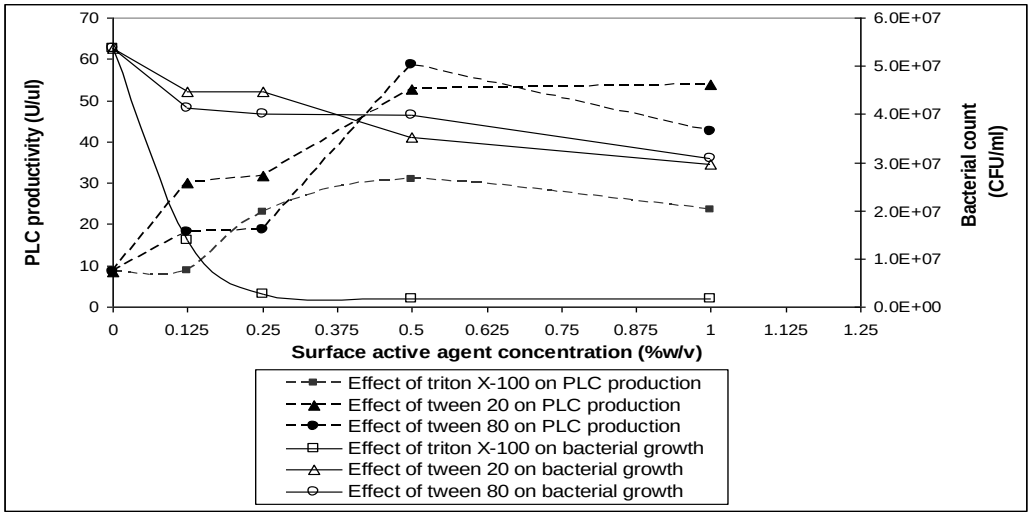


Figure 3. Effect of triton X-100, tween 20, tween 80 and sodium cholate on growth and PLC production by *Pseudomonas aeruginosa* D183.

a) Effect of triton X-100, tween 20, tween 80.



b) Effect of sodium cholate

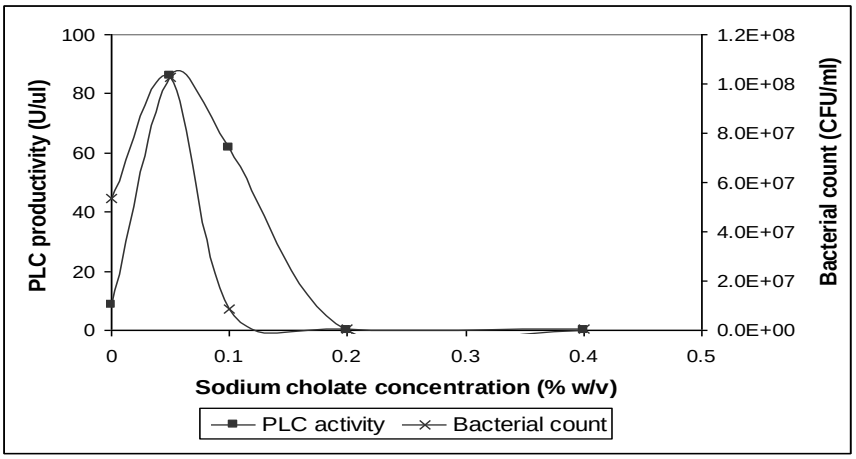
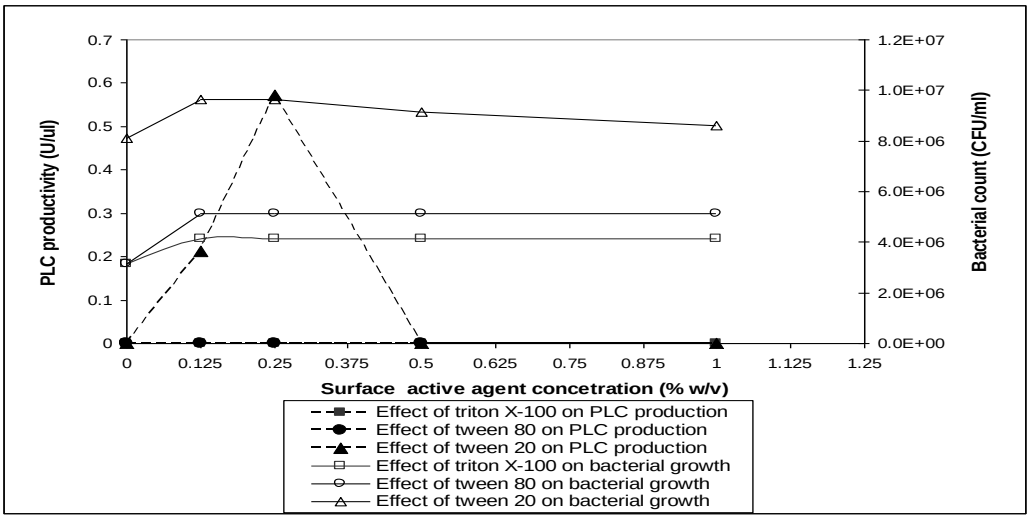


Figure 4. Effect of triton X-100, tween 20, tween 80 and sodium cholate on growth and PLC production by *Bacillus cereus* D101.

a) Effect of triton X-100, tween 20, tween 80



b) Effect of sodium cholate.

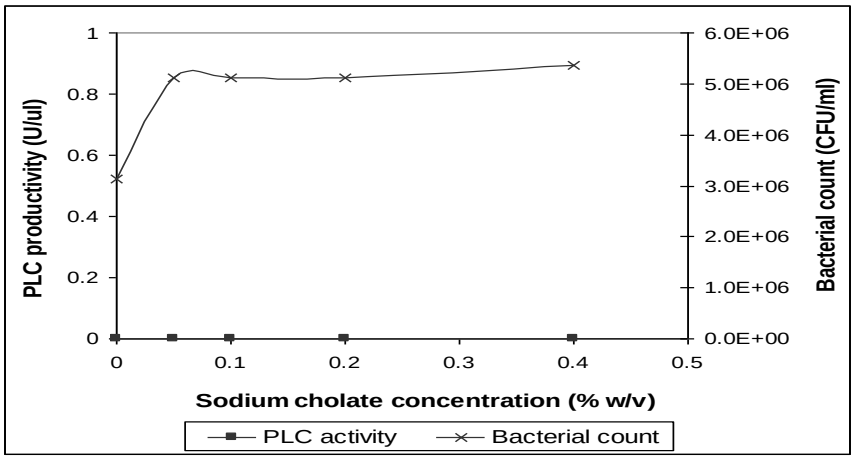


Figure 5. Effect of triton X-100, tween 20, tween 80 and sodium cholate on growth and PLC production by *Staphylococcus aureus* D173.

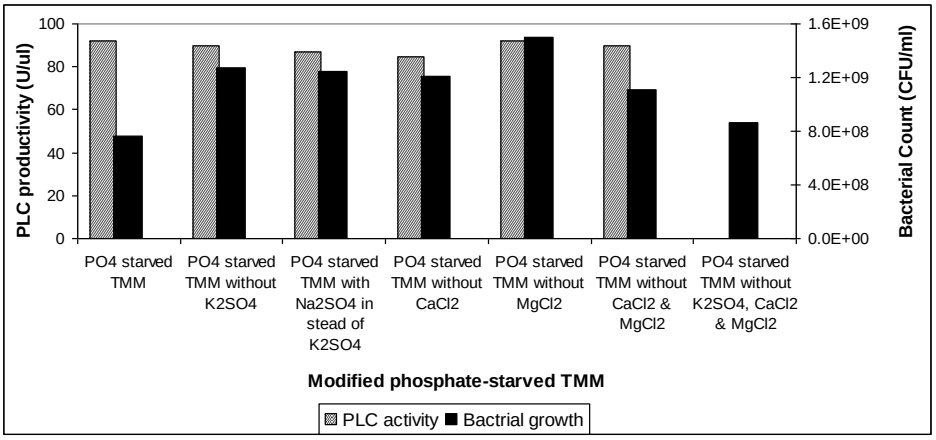
Effect of Different Metal Ions on the Production of PLC

1. Effect of Removal or Replacement of Metal Salt Components of Phosphate-Starved TMM

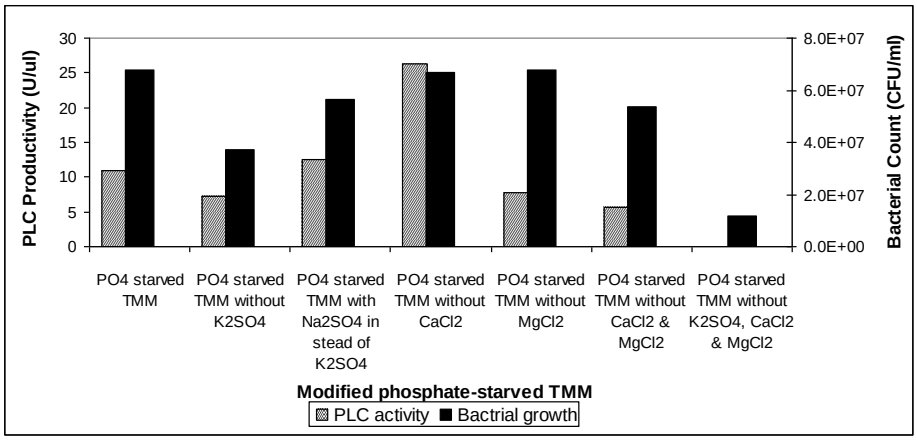
The effect of the metal salt components of phosphate-starved TMM on growth and PLC production from the test isolates was studied by removing them, one at a time, leaving only KH_2PO_4 unchanged, and measuring growth and PLC production in each case. So, in the first case, K_2SO_4 was removed, in the second, $CaCl_2$ was removed, the third, $MgCl_2$ was removed, the forth, both $CaCl_2$ and $MgCl_2$ were removed, and in the fifth case, K_2SO_4, $CaCl_2$ and $MgCl_2$ were removed (basal phosphate-starved TMM). In another experiment, K_2SO_4 was replaced with Na_2SO_4. The removal of K_2SO_4, $CaCl_2$, $MgCl_2$, both $CaCl_2$ and $MgCl_2$, or the replacement of K_2SO_4 by Na_2SO_4 showed a little effect on PLC production by ***Pseudomonas aeruginosa* D183**, although such removal increased its growth (Figure 6a). However, the removal of all three metal salts (basal phosphate-starved TMM) caused PLC production to drop to zero, yet no pronounced effect on growth was detected. With ***Bacillus cereus* D101,** unlike the case with *Pseudomonas aeruginosa* D183, removing K_2SO_4, replacing it with Na_2SO_4, or removing $MgCl_2$, $CaCl_2$ or both $CaCl_2$ and $MgCl_2$ caused a variable effect on both growth and PLC production by *Bacillus cereus* D101 (Figure 6b). Removal of K_2SO_4 caused a considerable decrease in both growth and PLC production while its replacement with Na_2SO_4 had a little effect on both parameters. PLC production by the test isolate, and not its growth, was obviously affected by the separate or combined removal of calcium and magnesium salts; where calcium removal caused more than two fold increase in PLC production, the separate removal of magnesium or the combined removal of calcium and magnesium salts decreased the enzyme production. The triple removal of potassium, calcium and magnesium ions drastically decreased bacterial growth and abolished PLC production. For

***Staphylococcus aureus* D183.** As mentioned earlier phosphate-starved TMM did not support PLC production by this test isolate although it potentiated bacterial growth. Interestingly, the removal of K_2SO_4 from such medium, its replacement with Na_2SO_4, or the separate removal of $CaCl_2$ or $MgCl_2$ relieved this inhibition and supported low levels of PLC production (Figure 6c). The highest enzyme production was observed upon the replacement of K_2SO_4 with Na_2SO_4. The combined removal of $CaCl_2$ and $MgCl_2$ did not enhance PLC production by the isolate, and neither did the combined removal of K_2SO_4, $CaCl_2$ and $MgCl_2$. In all cases, the resultant modified phosphate-starved TMM caused little reduction in bacterial growth.

***a) Pseudomonas aeruginosa* D183.**



***b) Bacillus cereus* D101.**



c) *Staphylococcus aureus* D173.

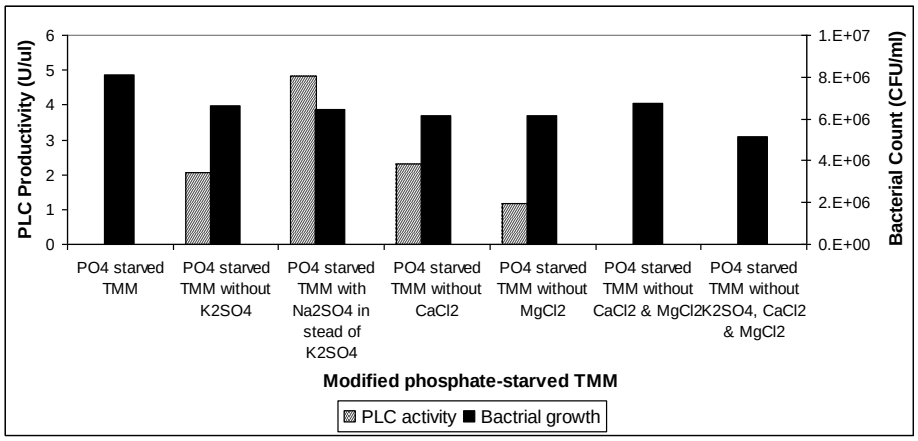
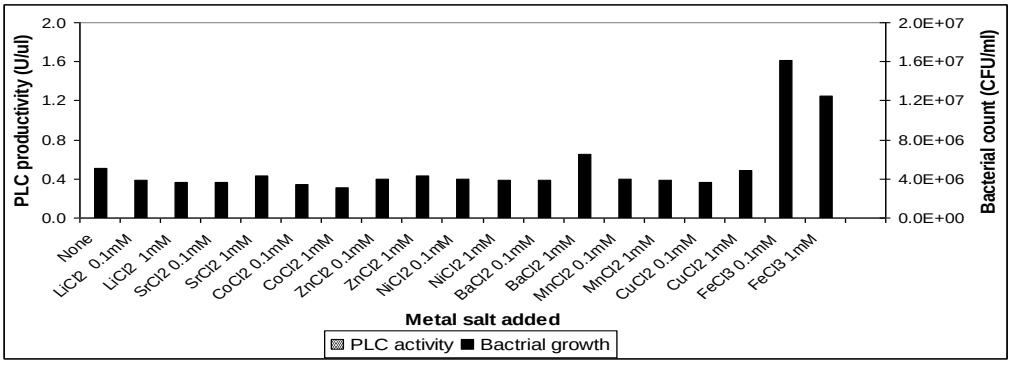


Figure 6. Effect of removal or replacement of metal salt components of phosphate-starved TMM on growth and PLC production by *Pseudomonas aeruginosa* D183, *Bacillus cereus* D101, and *Staphylococcus aureus* D173.

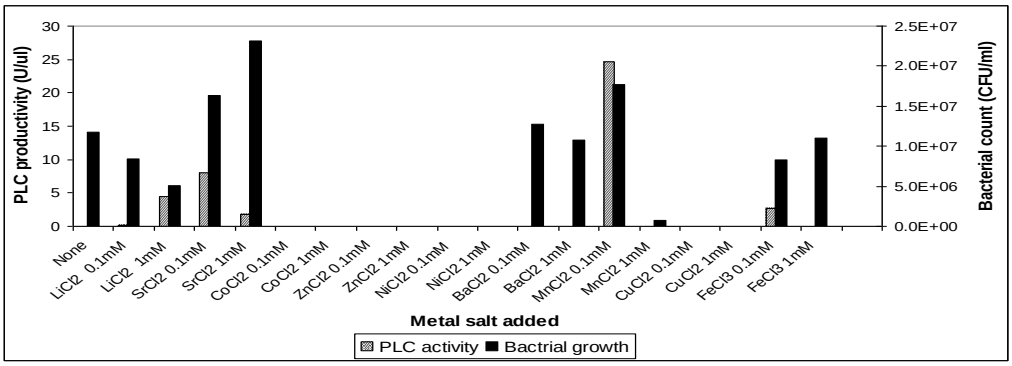
2. Studying the Effect of Addition of Other Metal Salts to Basal Phosphate-Starved TMM.

This was carried out by adding different metal salts to basal phosphate-starved TMM and measuring their effects on growth and PLC production. For ***Pseudomonas aeruginosa* D183,** when the chloride salts of different metals were added to basal phosphate-starved TMM, only $LiCl_2$ and $FeCl_3$ were able to restore some PLC production at the two concentration levels tested (Figure ***7***a). Nonetheless, the effect of these chloride metal salts on growth was variable; only ferric chloride at low concentration (0.1 mM) increased growth. The chloride salts of barium, lithium, and strontium had a little effect on growth while a sharp decline (≥70%) in growth was observed with cobalt, zinc and nickel chlorides. Both copper and manganese chlorides caused about 50% reduction in growth. However for ***Bacillus cereus* D101**, the addition of metal salts to basal phosphate-starved TMM was accompanied by PLC production only in case of $LiCl_2$ (1mM), $SrCl_2$ (0.1 and 1 mM), $MnCl_2$ (0.1 mM) and $FeCl_3$ (0.1 mM) (Figure 7b). On the other hand, the isolate was unable to grow when $CoCl_2$, $NiCl_2$, $ZnCl_2$ and $CuCl_2$ were added. For ***Staphylococcus aureus* D183**, none of the added metal salts was able to support PLC production from the isolate under the tested concentrations (Figure 7c). As per bacterial growth, ferric chloride showed high growth levels and still reasonable growth was obtained with other metal salts.

a) ***Pseudomas aeruginosa* D183.**



b) ***Bacillus cereus* D101.**



c) ***Staphylococcus aureus* D173.**

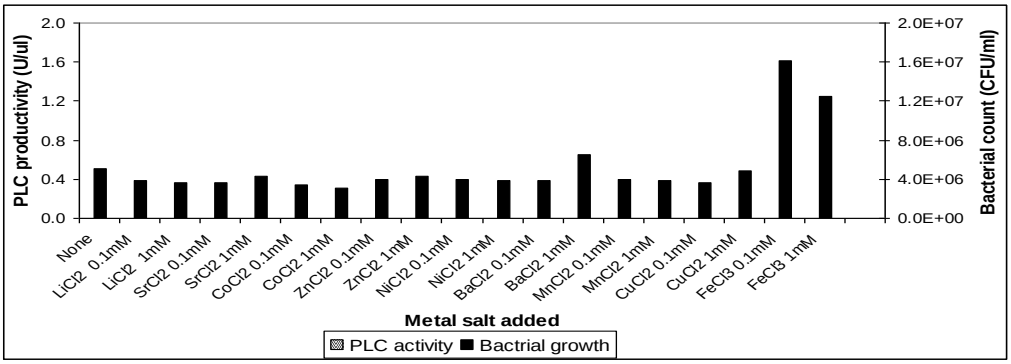


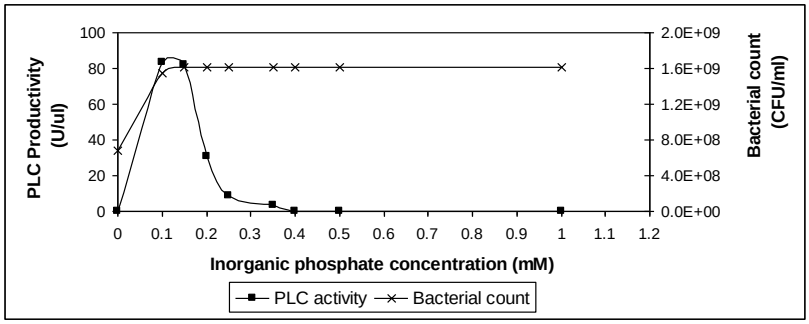
Figure 7. Effect of addition of metal salts to basal phosphate-starved TMM on growth and PLC production by *Pseudomonas aeruginosa* D183, *Bacillus cereus* D101, and *Staphylococcus aureus* D173.

In the forecoming experiments, for each isolate, the medium that gave maximum or comparable PLC production, compared to the other tested media, was used for studying the effect of other factors on production. Accordingly, phosphate-starved TMM was used for *Pseudomonas aeruginosa* D183, phosphate-starved TMM supplemented with 0.05% (w/v) sodium cholate for *Bacillus cereus* D101, and phosphate-starved TMM supplemented with 1.5% (w/v) BSA for *Staphylococcus aureus* D173.

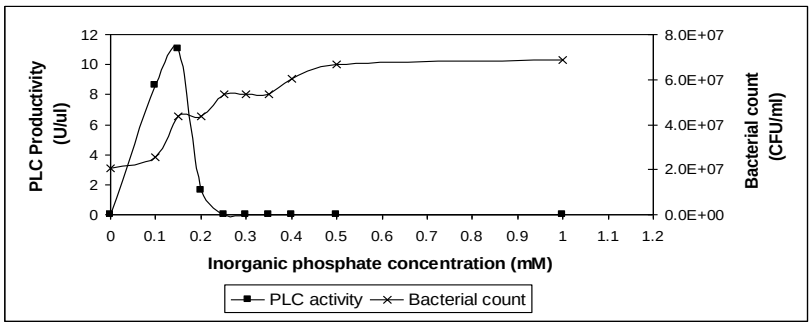
Effect of Inorganic Phosphate Concentration on the Production of PLC

As per ***Pseudomonas aeruginosa* D173 and *Bacillus cereus* D101**, as shown in Figure 8 (a) and (b), the optimum concentrations of inorganic phosphate for PLC production by the two isolates were 0.1 and 0.15 mM after which PLC production dropped sharply although considerable growth was detected. While for ***Staphylococcus aureus* D173**, high PLC production by the isolate was detected at inorganic phosphate concentrations in the range from 0.1 - 0.25 mM (Figure 8 c), and production decreased gradually by increasing the concentration until it reached zero at 1 mM concentration. On the other hand, growth increased with increasing inorganic phosphate concentration to reach its maximum level at 1 mM concentration.

a) ***Pseudomonas aeruginosa* D173 in phosphate-starved TMM.**



b) ***Bacillus cereus* D101 in phosphate-starved TMM supplemented with 0.05% sodium cholate.**



c) ***Staphylococcus aureus* D173 in phosphate-starved TMM supplemented with 1.5% BSA.**

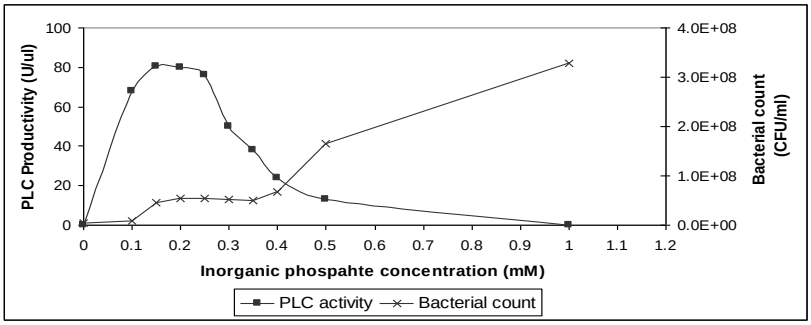
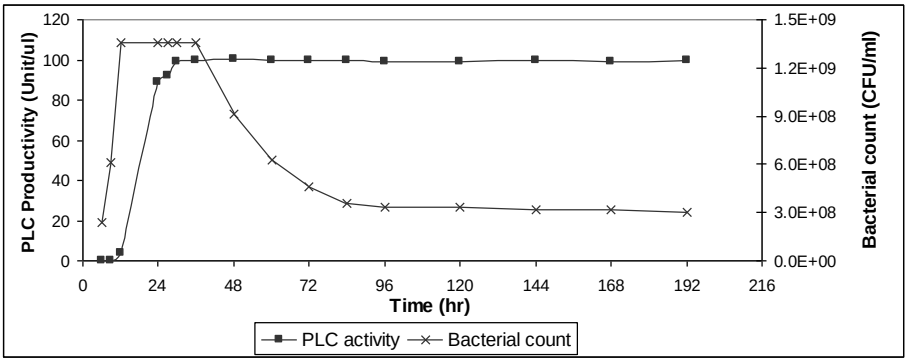


Figure 8. Effect of inorganic phosphate on growth and PLC production by the test isolates.

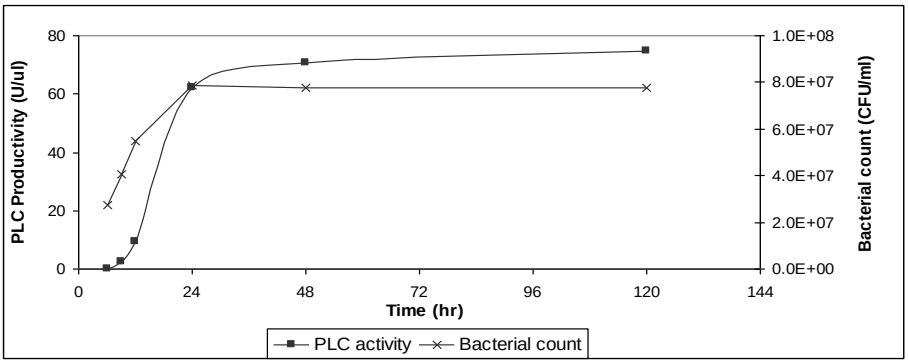
Effect of Incubation Period on the Production of PLC

For ***Pseudomonas aeruginosa*** **D183**, the results (Figure 9a) showed that after 12 h of incubation, cell growth reached its maximum and PLC production started to appear. Maximum enzyme production was achieved after around 30 h followed by a plateau, while cell growth showed a gradual decline after a 24 h stationary phase to reach a constant level after 84 h of incubation. While for ***Bacillus cereus*** **D101**, as shown in Figure 9b, bacterial cell growth reached its maximum after 24 h of incubation which was concomitant with the start of PLC production at high levels. Maximum PLC production was reached after 36 h. For both growth and PLC production plateau levels were observed after reaching the maxima. For ***Staphylococcus aureus*** **D173**, growth of the isolate maximized after 24 h and started to drop after 48 h of incubation. PLC production was recorded after 24 h and maximized after 36 h with no change in its level thereafter (Figure 9c).

a) ***Pseudomonas aeruginosa*** **D173 grown in phosphate-starved TMM**



b) ***Bacillus cereus*** **D101 in phosphate-starved TMM supplemented with 0.05% sodium cholate**



c) ***Staphylococcus aureus*** **D173 in phosphate-starved TMM supplemented with 1.5% BSA**.

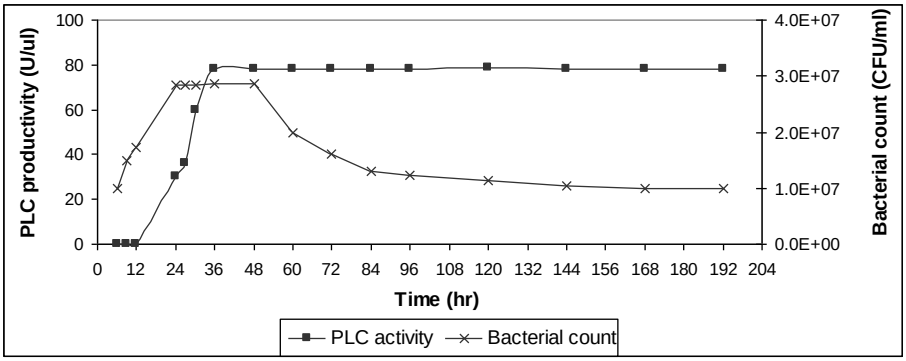
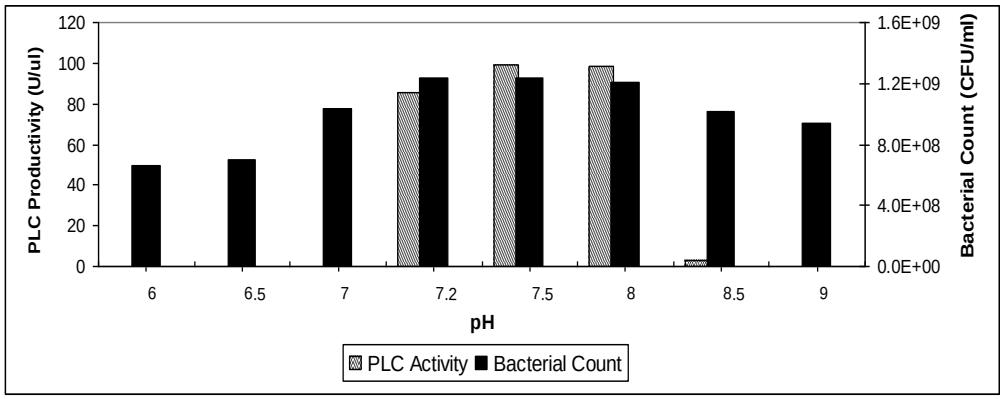


Figure 9. Effect of incubation period on growth and PLC production by the test isolates.

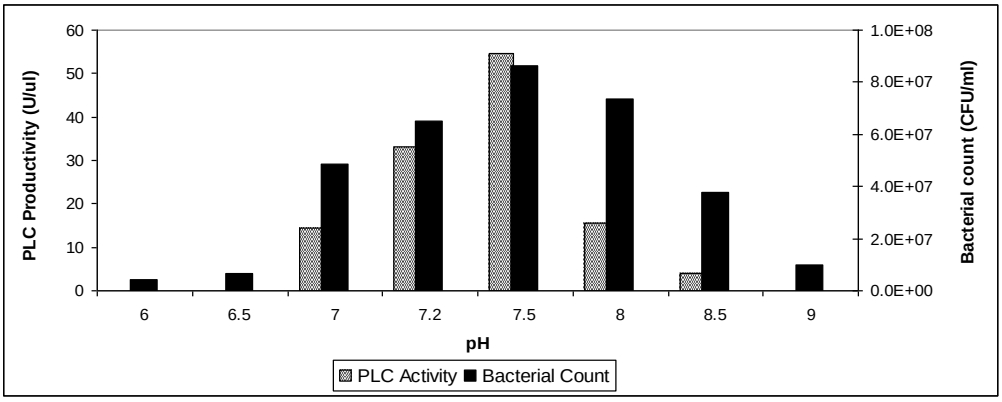
Effect of Initial pH on the Production of PLC

***Pseudomonas aeruginosa* D183** could grow at all the pH values tested, with maximum growth at pH 7.2, 7.5 and 8; whereas PLC production was detected only at pH values 7.2, 7.5, 8 and at a very low level at 8.5 (Figure 10a). ***Bacillus cereus* D101** showed reasonable cell growth, as well as PLC production at the tested pH values between 7 and 8.5 and maximum values for both parameters were at pH 7.5 (Figure 10b). Low cell growth levels with no PLC production were recorded at the other tested pH values. ***Staphylococcus aureus* D173** could grow at all the pH values tested; with maximum growth at pH values 7, 7.2 and 7.5 (Figure 10c). PLC production was detected only at pH values 7, 7.2, and 7.5.

a) *Pseudomonas aeruginosa* D173 grown in phosphate-starved. TMM.



b) *Bacillus cereus* D101 grown in phosphate-starved TMM supplemented with 0.05% sodium cholate.



c) *Staphylococcus aureus* D173 in phosphate-starved TMM supplemented with 1.5% BSA.

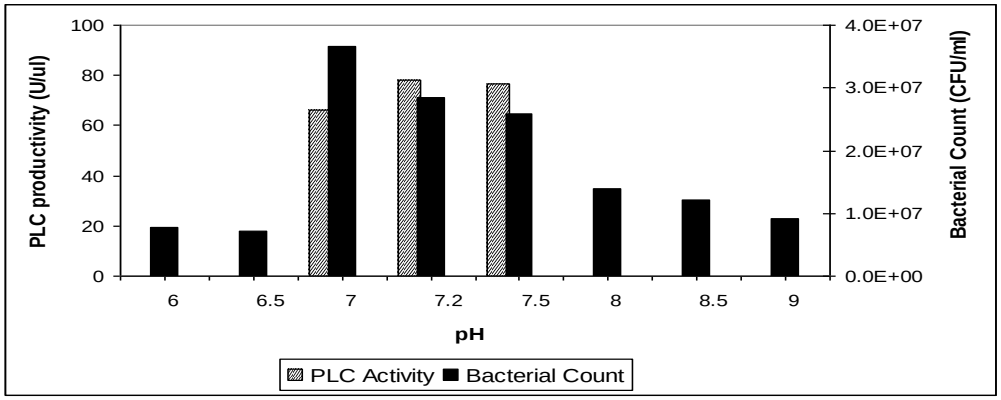
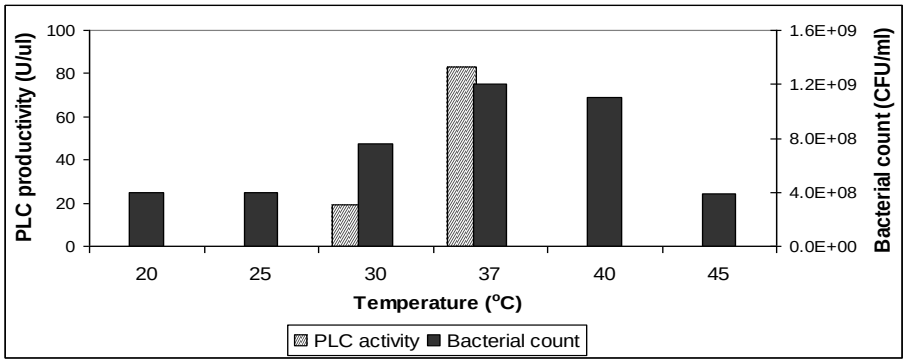


Figure 10. Effect of initial pH on growth and PLC production by the test isolates.

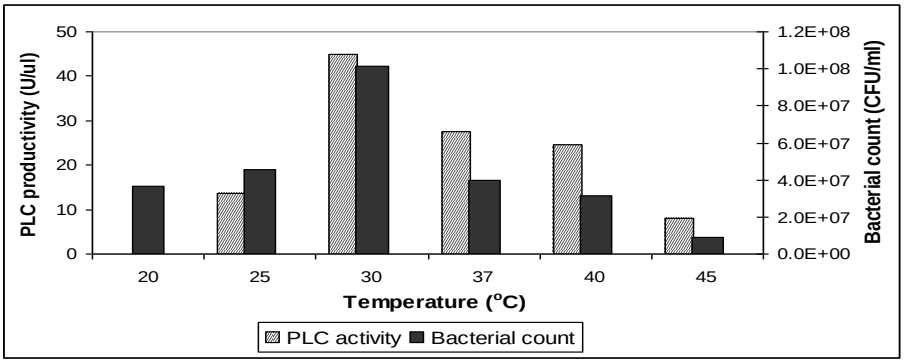
Effect of Incubation Temperature on the Production of PLC

***Pseudomonas aeruginosa* D183** could grow at all the tested temperatures while PLC production occurred only at 30 and 37°C. Maximum cell growth and PLC production were obtained at 37°C (Figure 11a). As per ***Bacillus cereus* D101**, although with different degrees, all the tested temperatures could promote growth of the isolate and maximum growth and PLC production were obtained at 30°C (Figure 11b). With the exception of 20°C, PLC production was detected at all tested temperatures. ***Staphylococcus aureus* D173** showed considerable growth with PLC production only at incubation temperatures 25, 30 and 37°C. However, at 20, 40 and 45°C low levels of growth with no PLC production were detected (Figure 11c).

a) *Pseudomonas aeruginosa* D183 grown in phosphate-starved TMM.



b) *Bacillus cereus* D101 in phosphate-starved TMM supplemented with 0.05% sodium cholate.



c) *Staphylococcus aureus* D173 grown in phosphate-starved TMM supplemented with 1.5% BSA.

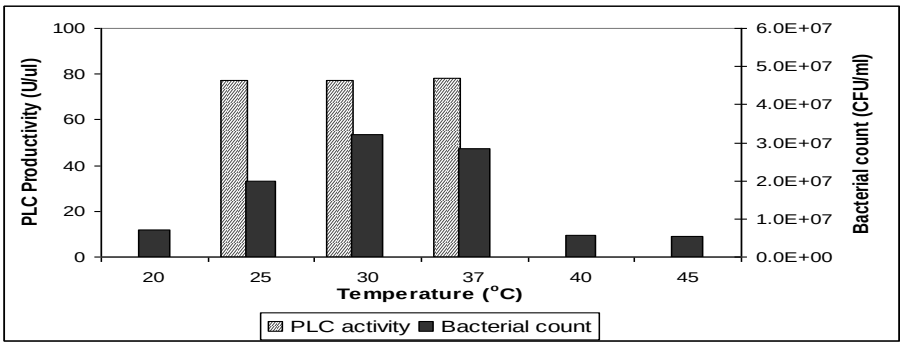


Figure 11. Effect of incubation temperature on growth and PLC production by the test isolates.

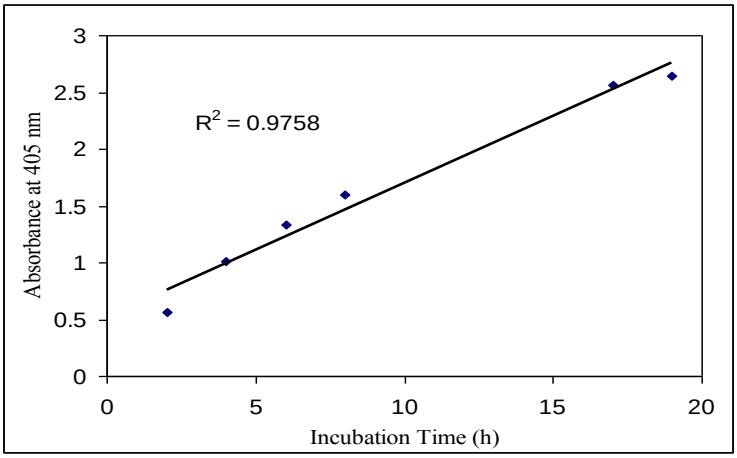


Figure 12. Relationship between incubation time and absorbance of yellow color liberated by a constant amount of the clear supernatant of a selected isolate using chromogenic assay of PLC activity.

4. Discussion

A variety of pathological specimens was collected to recover clinical isolates and evaluate their ability to produce phospholipases. The goal was to find strong PLC producing isolates from different bacterial species, then to evaluate the effect of different factors on the productivity of PLC by a few of them. The recovered bacterial clinical isolates, 205 isolates, were primarily classified by Gram staining and microscopical examination. Of these isolates, 103 were Gram-negative rods, 60 were Gram-positive cocci with the characteristic morphology of genus *Staphylococcus*, 22 isolates were Gram-positive diplococci, 12 isolates were Gram-negative coccobacilli, 5 were Gram-positive thick bacilli with the characteristic morphology of genus *Bacillus*, 2 isolates were Gram-positive cocci arranged in chains, and one isolate was Gram-positive cocci arranged in tetrads. Primary screening for phospholipase production was performed by the egg-yolk agar plate method described by Price *et al.* (1982) and the level of phospholipase production was determined as described by Dagdeviren *et al.* (2005). About 25% of the screened isolates were able to produce phospholipase; of these positive isolates 64% were very strong producers, 12% were strong producers, 19% were mild producers, and 5% were weak producers. Phospholipase production prevails among Gram-negative rods, but not among Gram-negative coccobacilli, and among Gram-positive thick rods and Gram-positive cocci in clusters but not among Gram-positive diplococci, cocci in chains, or in tetrads. Phospholipase production is also common among yeasts.

Of the 43 phospholipase producing isolates obtained in the primary screening, 28 were considered very strong phospholipase producers. Therefore, these isolates were subjected to a more quantitative secondary screening test for characterizing their PLC productivities using the chromogenic assay in which NPPC was used as a substrate for the enzyme. Not all the very strong phospholipase producing isolates obtained from primary screening exhibited the same high PLC productivities when assayed by the chromogenic assay method. This may be attributed to the fact that the formation of opacity on egg-yolk plates may be caused by numerous substances (Flieger *et al.*, 2000). The use of NPPC as an artificial substrate to detect PLC activity is very common (Flieger *et al.*, 2000). NPPC is cleaved by PLC to phosphocholine and p-nitrophenol. The optical density of the yellow compound p-nitrophenol (p-NP) is determined colorimetrically at 405 nm. Since the rate of hydrolysis of NPPC by PLC is very low in aqueous media, 60% sorbitol was added to the reagent to improve the hydrolytic rate. Such an effect of sorbitol may be related to an improvement in the characteristics of the reaction medium; a hydrophobic environment surrounding NPPC and PLC is suspected to exist as a result of hydration of sorbitol added to the reaction medium (Kurioka and Matsuda, 1976). In this study, NPPC was used at a low concentration (10 mM), since even at this concentration the time course curve was always linear during the period of measurement. The reaction mixture also included $ZnCl_2$, since the maximal catalytic activity of PLC is obtained in the presence of 10^{-4} M $ZnCl_2$ as this divalent metal cation, at the specified concentration, increases the V_{max} of the catalytic reaction (Kurioka and Matsuda, 1976).

From each morphological class tested (Gram-negative rods, Gram-positive thick rods arranged in chains, and Gram-positive cocci arranged in clusters), the isolate that gave the maximum PLC production, as well as maximum specific productivity, was selected for further studies. These isolates were identified, according to Burgey's Manual of Systemic

Bacteriology (1989), with the help of Baird (1996) for the selected Gram-positive cocci in clusters isolate, and Govan (1996) for the selected Gram-negative rods isolate. The isolates were identified as *Pseudomonas aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus*, respectively.

After the biological entities for PLC production from different species have been selected and identified, the next task was to study the effect of different factors on PLC production. A basal synthetic medium that contains the basic requirements for bacterial growth was used. Tris Minimal Medium (TMM) contained ammonium chloride as an inorganic source of nitrogen; glucose as a source of carbon; tris-HCl as a buffer; $MgCl_2$, $CaCl_2$ and K_2SO_4 as mineral sources; and KH_2PO_4 at 10 mM concentration as a phosphate source (Norte *et al.*, 2003). When tested, TMM was able to support growth of the selected isolates but was unable to support PLC production by them. Testing modifications of TMM, including phosphate-starved TMM which contained only 0.1 mM KH_2PO_4, nitrogen-starved TMM which contained one tenth the amount of ammonium chloride in TMM, and magnesium-starved TMM at which the concentration of $MgCl_2$ is 1/1000 that in TMM and $CaCl_2$ was omitted (Norte *et al.*, 2003), the problem appeared to be in the phosphate concentration. Only phosphate-starved TMM was able to support PLC production from *Pseudomonas aeruginosa* D183 and *Bacillus cereus* D101; however production of PLC from *Staphylococcus aureus* D173 needed further adjustment of conditions. Phosphate-limiting conditions induce the expression of PLC as well as several other phosphate-regulated products such as alkaline phosphatase, and phosphate transport proteins (Titball, 1993). This has led many authors to suggest that the major role of PLC, along with phosphatases, is to scavenge phosphate by degrading host phospholipids (Pritchard and Vasil, 1986; Guddal *et al.*, 1989; Ostroff *et al.*, 1989; Hansen *et al.*, 1992; Vasil, 2006). The absence of one of these phosphate-regulated components may impede the ability of the organism to acquire phosphate during phosphate limitation (Titball, 1993).

Based on the above mentioned discussion, phosphate-starved TMM was selected as the basal medium for studying the effect of different factors on PLC production by the selected isolates. In an attempt to study the effect of the carbon source and its concentration on PLC production, the concentration of glucose in phosphate-starved TMM was increased 5 folds and 10 folds. It was also replaced by other carbon sources at the same three concentrations. These tests revealed that glucose was the most suitable carbon source for PLC production; though its optimal concentration varied among the tested isolates; for *Pseudomonas aeruginosa* D183, maximal PLC production occurred at 11 mM glucose (the concentration used in phosphate-starved TMM) rather than the higher glucose concentrations (55 and 110 mM) (Figure 1a). These findings come in agreement with the results obtained by Stinson and Hayden (1979) who studied the conditions necessary for the secretion of phospholipase C by *Pseudomonas aeruginosa* and recorded that maximum PLC production, in tryptose minimal medium, occurred when the concentration of glucose was 10 mM. On the other hand, *Bacillus cereus* D101 and *Staphylococcus aureus* D173 needed higher glucose concentrations (110 mM) for maximal PLC production (Figure 1b and c).

Although the addition of organic nitrogen sources to phosphate-starved TMM supported excellent growth of the test isolates, an inhibition of PLC production could be detected in all such preparations, except those containing BSA at 1.5% and peptone at 0.5% for *Pseudomonas aeruginosa* D183, BSA at 0.5, 1 and 1.5% for *Bacillus cereus* D101 and *Staphylococcus aureus* D173. For *Staphylococcus aureus* D173, BSA was the only additive that could support high levels of PLC production. This inhibition of PLC production by the addition of most protein sources may be attributed to the high content of organic phosphates present in these protein sources (Hasegawa *et al.*, 1982). These organic phosphates are hydrolyzed by the periplasmic enzyme alkaline phosphatase (Cheng *et al.*, 1970) into inorganic phosphates which in turn inhibit PLC production. This inhibitory effect did not accompany the addition of BSA since it is free from organic phosphates (Enomoto *et al.*, 2008); besides, BSA is known to cause inactivation of alkaline phosphatase activity (Henneman *et al.*, 1955;Foster and Bannister, 1976). These results may suggest that BSA has a positive effect on the production of PLC from the test isolates. The results also indicate that PLC production by the test isolates is favored at nutrient limiting media and that a low growth rate favors PLC production (Bjorn *et al.*, 1979; Hirata *et al.*, 1995; Ouhib *et al.*, 2006).

The addition of the tested surface active agents to phosphate-starved TMM did not have a noteworthy effect on growth or PLC production from *Pseudomonas aeruginosa* D183 or *Staphylococcus aureus* D173, at the tested concentrations (Figures 3,5). Conversely, the addition of triton X-100, tween 20 and tween 80 decreased growth and increased PLC production by *Bacillus cereus* D101. Sodium cholate addition supported high levels of growth and PLC production by the *Bacillus cereus* isolate only at low concentrations (0.05 and 0.1% w/v for PLC production and 0.05% for growth), while at higher concentrations (0.2 and 0.4%), both growth and production leveled off (Figure 4). According to Chin and Watts (1988) the

increase in PLC production caused by the addition of surface active agents is due to the perturbation of the membrane system of the isolate resulting in increased production and release of PLC. Morotomi *et al.* (1990) and Vulevic *et al.* (2004) reported that these results could be of biological significance since bile salts are present in the lumen of the colon at the concentration range found to enhance PLC production and thus DAG formation by fecal bacteria. DAG is a normal physiological activator of protein kinase C, an enzyme that plays a key role in growth control and tumor promotion, therefore, DAG produced by the intestinal microflora might stimulate growth of colonic epithelial cells. Thus an interaction between dietary lipids, bile salts, and PLC production by specific bacteria in the intestinal lumen could contribute to the risk of colon cancer development in humans.

The effect of metal ions on the growth and production of PLC by the selected isolates was tested on two steps; first, the effect of the stepwise removal of the metal salt components, with the exception of KH_2PO_4, from phosphate-starved TMM. The effect of replacement of K_2SO_4 with Na_2SO_4 at the same concentration was also tested. Second, was the effect of addition of different metal ion salts to phosphate-starved TMM from which all its metal ion components have been removed leaving only KH_2PO_4, named basal phosphate-starved TMM. For *Pseudomonas aeruginosa* D183, the removal of metal salts from phosphate-starved TMM and the replacement of K^+ by Na^+ did not affect PLC production, nonetheless, production was lost, although considerable growth was detected, when all three metal salts (KSO_4, $MgCl_2$, $CaCl_2$) were removed leaving only $KHPO_4$ (Figure 6a). This proves that cations are required for PLC secretion by cultures of *Pseudomonas aeruginosa* (Stinson and Hayden, 1979). Only Li^+ and Fe^{3+} ions could restore some PLC productivity when added to basal phosphate-starved TMM (Fig. 7a).

For *Bacillus cereus* D101, PLC production was lost only when all three metal ion salts (KSO_4, $MgCl_2$, and $CaCl_2$) were removed; however, PLC production increased, without an increase in bacterial growth, when $CaCl_2$ was omitted (Figure 6b). The addition of Co, Ni, Zn and Cu chlorides, at the tested concentrations, to basal phosphate-starved TMM completely inhibited the growth of the microorganism. Similar results about the toxicity of these metal ions on growth of *Bacillus* sp. were reported by several researchers including (Babich and Stotzky, 1978; Freitas et al., 2008; Kamala-Kannan et al., 2008). Although, the mechanisms responsible for the toxicity of these metal ions are essentially undefined, it has been suggested that they may interfere with the metabolism of Mg^{2+} (Babich and Stotzky, 1978). On the other hand, the addition of Li, St, Mn and Fe chlorides could restore some PLC production by the *Bacillus cereus* isolate (Fig. 7b). No PLC production was detected when phosphate-starved TMM, phosphate-starved TMM without both $CaCl_2$ and $MgCl_2$, phosphate-starved TMM without the three metal salts KSO_4, $MgCl_2$, and $CaCl_2$ were used with *Staphylococcus aureus* D173. When K_2SO_4 was omitted from phosphate-starved TMM, replaced with Na_2SO_4, or when $CaCl_2$ or $MgCl_2$ were omitted from such medium, low levels of PLC production were detected (Figure 6c). None of the metal salts tested by addition to basal phosphate-starved TMM was able to support PLC production by this isolate (Fig. 7c).

The production of PLC by the three tested isolates was found to be dependent on the level of inorganic phosphate in the production medium. The optimum levels were found to be 0.1 and 0.15 mM KH_2PO_4 for *Pseudomonas aeruginosa* D183 and *Bacillus cereus* D101, and 0.1-0.25 mM KH_2PO_4 for *Staphylococcus aureus* D173. At higher concentrations a sharp decline in PLC production occurred. The phosphate regulation of PLC production has been previously reported by many workers including (Pritchard and Vasil, 1986; Ostroff *et al.*, 1989; Hansen *et al.*, 1992; Kaenjak *et al.*, 1993;Gabdrakhmanova *et al.*, 2002). This suggests that PLC may be a component of a phosphate-scavenging mechanism (Stinson and Hayden, 1979; Vulevic *et al.*, 2004). Under cultural conditions where inorganic phosphate has been depleted, the PLC genes become derepressed and synthesis and secretion are initiated. The nutrient requirement for phosphate is subsequently satisfied by the release of phosphomonoesters from phospholipids by the action of PLC, followed by the liberation of free phosphate by the periplasmic enzyme alkaline phosphatase (Cheng *et al.*, 1970). This has led more than a few authors to surmise that the major purpose of PLC, along with phosphatases, is to scavenge Pi by degrading host phospholipids. While this may indeed be of benefit to the PLC producing organism in a phosphate-limiting environment, it is a very limited perspective *vis-à-vis* the real potential of this virulence determinant. Vasil (2006) has put forward a scenario whereby this virulence determinant, through its ability to generate another moiety from phospholipids (choline phosphate), would provide much more than just a single ion (i.e. Pi) for the survival of the bacteria. First, the organism can utilize choline phosphate as a sole source of carbon, nitrogen and Pi. Consequently, it would be possible for this organism to survive solely on phospholipids for these needs if it is expressing PLC. A further benefit of choline phosphate is that it will enable the organism to survive and grow in high osmolarity environments. Choline, once it is generated from choline phosphate by a phosphatase, can be readily taken up and converted to the osmolyte, glycine

betaine. Under conditions of high osmolarity (e.g. lungs of CF patients for *Pseudomonas aeruginosa*) the ability of the organism to generate choline, and convert it to glycine betaine, through the hydrolysis of phospholipids could allow it survive in this hostile environment. Other organisms that do not produce PLCs would be unable to survive in such a harsh milieu where phospholipids might be the only source of choline.

The test isolates secreted PLC during late logarithmic and early stationary growth phases. There is precedence for this production pattern recorded by Stinson and Hayden (1979) for *Pseudomonas aeruginosa* in tryptose minimal medium, Durban *et al.* (2007) for *Bacillus cereus* in Luria Bertani broth, Marques *et al.* (1992) for *Staphylococcus aureus* PI-PLC in dialysate of proteose peptone broth at both pH 5.0 and 7.4, and Coffey *et al.* (1996) for *Listeria monocytogenes* in a chemically defined minimal medium. The fact that most enzyme secretion systems in bacteria have been shown to be regulated by either catabolite or end product repression (Glenn, 1976; Stinson and Hayden, 1979) suggested that the delay in the appearance of PLC was because its synthesis was repressed by some components in the culture medium. It may well be that limiting growth substrates at the late logarithmic and early stationary phases stimulate the expression of PLC (Coffey *et al.*, 1996). The increase in the activity of PLC in the medium followed the rate of bacterial growth through the logarithmic phase and was leveled off in the late stationary phase. This type of fermentation was classified as growth associated (Shiloach *et al.*, 1973). The kinetics of PLC production might indicate that the enzyme is not associated with autolysis of the cells and that bacterial phospholipids probably are not hydrolyzed by the extracellular enzyme in intact bacterial structures (Shiloach *et al.*, 1973). Moreover, the finding that PLC activity appeared to be strongly related to cell density and as cultures entered the exponential phase activity increased in a linear fashion over time supports the possibility that PLC expression is likely under the control of quorum sensing systems (Glessner *et al.*, 1999; Dong *et al.*, 2002).

The optimum pH for PLC production by the test isolates was found to be 7.2 and/or 7.5. This data comes in agreement with the data reported by Stinson and Hayden (1979) who had studied of the effect of pH on PLC production by *Pseudomonas aeruginosa* and indicated an optimum range of pH 7.0 to 8. Good growth of *Pseudomonas aeruginosa* D183 was detected over the pH range tested (6-9). Similar results were obtained by Wolski *et al.* (2006) who recorded good growth of *Pseudomonas* sp. cells at pH values within a range of 6 to 8 in a glucose containing minimal salt medium. However, PLC production occurred only at pH values 7.2, 7.5, 8 and at a very low level at 8.5. This indicates that extremes of pH may be an inhibitory factor for PLC production by this isolate (Vasil, 2006). For *Bacillus cereus* D101, very low levels of growth and no PLC production were recorded at pH values 6, 6.5 and 9. The results about the growth of the isolate are not in line with the earlier findings that growth of *Bacillus cereus* was possible in the pH range 4.5-9.5 (Sutherland and Limond, 1993). However, the results about PLC production by the same isolate are in line with the enterotoxin production results recorded by Beattie and Williams (2002), who reported that the amount of enterotoxin produced by the steady state cultures of *Bacillus cereus* was pH-dependent. The greatest amounts of toxin detected were at pH 7 and 8, while at the pH extremes examined (pH 5 and pH 9), very low levels of toxin were detected. As per *Staphylococcus aureus* D173, PLC production occurred only at pH values 7, 7.2 and 7.5 which were the same pH values at which considerable growth occurred. These results come opposing to those obtained by Marques *et al.* (1992) on the production of enzymatically active PI-PLC by *Staphylococcus aureus*. They reported that lowering the pH of *S. aureus* cultures from 7.0 to 5.4 progressively increased the yield of PI-PLC. And that the final yield of PI-PLC was at least five-fold greater when the initial culture pH was 5.4 compared with 7.0. They added that low pH enhanced PI-PLC activity recovered from two *S. aureus* strains capable of high PI-PLC production, but not from a strain producing little PI-PLC.

Regarding production temperature, the optimum temperatures for PLC production by the test isolates were 37˚C for *Pseudomonas aeruginosa* D183, 30˚C for *Bacillus cereus* D101 and 25-37˚C for *Staphylococcus aureus* D173. Similar results were reported by Coffey *et al.* (1996) for PLC from *Listeria monocytogenes*, where maximum PLC production was at 30-37˚C. The maximum production of protease and lipase by *Pseudomonas aeruginosa* occurred at the same temperature (37˚C) as recorded by Kanwar *et al.* (2002) and Abd Rahman *et al.* (2005), respectively. Similarly, Agata *et al.* (2002) and Nilegaonkar *et al.* (2007) reported that the optimum production temperature for emetic toxin and protease, respectively, from *Bacillus cereus* was 30˚C. Herten *et al.* (2008) has also reported that the production of enterotoxin by *Staphylococcus aureus* occurred at temperatures between 20 and 37˚C.

In conclusion, the results presented herein provide evidence that media composition, nutrient availability, inorganic phosphate concentration, pH, and temperature all have a significant effect on microbial PLC production and such effect is bacterial species dependant.

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STRUCTURAL AND ULTRASTRUCTURAL ALTERATIONS IN MAMMALIAN ADRENAL CORTEX UNDER INFLUENCE OF STEROIDOGENESIS INHIBITOR DRUG

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Abstract: The risk of adverse human health effects due to endocrine-disrupting chemicals is of growing concern. In recent years, ketoconazole, an imidazole derivative has been developed and currently used in the medical fields as an anti-fungal and steroidogenesis inhibitor drug. The present study aimed to investigate the influence of ketoconazole in the structural and ultrastructural characteristics of albino rat adrenal cortex. Twenty adult male rats weighing 150-200 g. were divided into two even groups; group I were *i.p.* injected with 10mg/100g.b.wt. of ketoconazole dissolved in *1ml* physiological saline solution in a daily manner at 9am for 15 days. Whereas, group II were injected with *1ml* saline solution in the same manner. *Histologically*, adrenal cortex of treated rat displayed hypertrophy. Glomerulosa, fasciculata and reticularis cells were loaded with lipid droplets of variable sizes, occupying almost the cytoplasm thus displacing the nuclei eccentrically, which showed signs of pyknosis, karyorrhexis and karyolysis. *Ultrastructurally*, the three cortical zones displayed the presence of hypertrophied mitochondria filled with tightly packed tubular cristae, whereas the others having cavitaion results in a complete loss of cristae, and mitochondria are identified by the remainder cristae adjacent to the inner boundaries of the limiting membrane, in addition to extensive accumulation of variable sized lipid droplets and nuclei showing pyknosis and karyolysis. In conclusion, it is noticed that the destructive impacts of ketoconazole on the adrenocortical cells reflected on their functions leading to much deficiency in their performance. So, it should be taken in consideration and great concern that this drug must be utilized under restricted precautions in the medical fields to protect the human health from its hazardous impact.

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1. Introduction:

The risk of adverse human health effects due to endocrine-disrupting chemicals is of growing concern. Despite that, the adrenal gland is an important organ yet it is neglectable in relation to effects of endocrine disruptors (Harvey *et al.,* 2007; Diamanti-Kandarakis *et al.,* 2009). This importance is being out from the fact that the adrenocortical zones synthesize and secrete steroid hormones, which fall into three major categories; mineralcorticoid, exemplified by aldosterone which is secreted by zona glomerulosa. Aldosterone is an important regulator of salt homeostasis and fluid balance and it is a major control unit of acid/base balance (Bielohuby *et al.,* 2007). While, zona fasciculata secrets gluco-corticoid, exemplified by cortisol, which is essential for life since it has a major role in responding to environmental stimuli; it decreases protein synthesis, thereby increasing the circulating level of amino acids; it elevates blood glucose by stimulating the enzymes involved in gluconeogenesis in the liver and it mobilizes fatty acids and glycerol from adipose cells. It has also anti-inflammatory effects (Fawcett and Jensh, 2002; Campbell, 2005). By zona reticularis, small amounts of androgens are secreted. The two principal adrenal androgens are; androstenedione and dehydroepi-androsterone, which is far less potent than testosterone and has little physiological significance. Both hormones can serve as substrates for the conversion into testosterone and estradiol (Fawcett and Jensh, 2002; Keegan and Hammer, 2002). The hormone secretions of the adrenal cortex is dependent on steroidogenic cytochrome P450 (CYP) enzymes which possibly can be the target of endocrine-disrupting chemicals affecting them (Ohlsson *et al.,* 2010).

One of the groups that effects CYPs is the imidazoles, which are medically used as antifungal treatment. Of the imidazoles in current clinical use, ketoconazole which is a broad-spectrum anti-fungal and steroid hormones biosynthesis inhibitor drug (Marty *et al.,* 2001; Schimmer and Parcker, 2006). It has great activity against a variety of yeast, dimorphic fungal, fungal infections of the gastro-intestinal tract, dermatophytic infections of the skin and has been widely used in immunocompromised patients such as those with AIDS or those on chemotherapy (Vertzoni *et al.,* 2006). Also, it has anti-inflammatory activity, it may prevent the development of acute respiratory distress syndrome

and acute lung injury in critically ill patients (Wiedemann *et al.,* 2000).

Ketoconazole is an effective inhibitor of adrenal and gonadal steroidogenesis, primarily because of its inhibition of the activity of CYP17. At even higher doses, it also inhibits CYP11A1, effectively blocking steroidogenesis in all primary steroidogenic tissues (Cohen *et al.,* 2000; Schimmer and Parcker, 2006).

In the field of medicine, the great ability of ketoconazole to inhibit mineralcorticoid synthesis is used for palliative treatment of primary hyper-aldosteronism (Lionakis *et al.,* 2008). Also, in a treatment strategy of resistant and hyper-cortisolemic depressive patients as an inhibitor of glucocorticoid synthesis (Brown *et al.,* 2001; Dvorak, 2011). As well as, in treatment of ACTH-secreting adenomas, palliative treatment of Cushing disease, adrenal tumors, adrenocortical carcinoma and ectopic corticotrophin production by small-cell lung carcinoma or carcinoid tumors (Gordon, 2007; Lionakis *et al.,* 2008).

Ketoconazole as inhibitor of androgen has been used for treatment of prostate cancer with promising results (Peehl *et al.,* 2001; Kinobe *et al.,* 2006; Liebertz and Fox, 2006).

Also, physicians use high-dose ketoconazole in women with advanced breast cancer resistant to conventional chemotherapy and for ovarian hyperandrogenism syndrome, including polycystic ovarian syndrome and hyperthecosis with considerable improvement in acne, hirsutism, and amenorrhea (Lionakis *et al.,* 2008).

In experimental animals, impact of ketoconazole has been studied in some biological aspects on different body organs rather than the adrenal gland (Rodriguez and Buckholz, 2003; Braddock, 2003; Amin and Hamza, 2005; Furukawa *et al.,* 2008).

It is clearly noticed from the previous literature, that ketoconazole has been widely utilized in the medical fields for the treatment of different types of diseases. But, unfortunately there is no attention for the influence of its administration on the adrenal cortex which is responsible for synthesis and secretion of different steroid hormones. Thus, the present study aimed to throw light on the influence of ketoconazole on adrenal cortical tissues from the histological and ultrastructural point of view

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2. Materials and Methods:

2.1. Experimental animals

Twenty male Swiss albino rats (*Rattus norvegicus*) ranging in weight from 150-200g., acquired from Schistosoma Biological Supply Program (SBSP) Theodor Bilharz Research Institute, were housed in clear plastic cages (2 animals/cage) with wood chips as bedding and given pellet rodent diet, in addition of milk and water *ad-libitum*. They were kept under controlled environmental conditions, including a temperature of 25^0C and a 12-h light/darkness cycle.

2.2. Drug used

Ketoconazole is a synthetic imidazole of oral broad-spectrum antifungal agent (Vertzoni *et al.,* 2006; Dantas *et al.,* 2010). It is sold under trade name; Nizoral® as a tablet of 200 mg Ketoconazole which is manufactured by JANSSEN-CILAG Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse, Belgium.

2.3. Experimental design

The rats were randomly divided into two even groups; group I, were *i.p.* injected with 10mg/100g.b.wt. of *ketoconazole* dissolved in *1 ml* of physiological saline solution in a daily manner for 15 days. Whereas, group II was kept as a control group and were injected with *1ml* of physiological saline solution in the same manner.

This selected dose of ketoconazole and the route of administration have been previously used in different researches (O`Connor *et al.,* 2002; Amin and Hamza, 2005; Amin, 2008).

2.4. Histological preparations

The excised adrenal glands were fixed in Bouin`s fluid for 24 hours, then subjected to the normal procedures for paraffin sectioning. Sections, of 4-6 µm were stained with haematoxylin & Eosin, dehydrated, cleared in xylene and mounted in DPX. The stained sections were examined and photographed by light microscopy (BX-40 Olympus), fitted with 4x - 40x objective lenses with an adjustable numerical aperture (3.3). Images were captured using camera (Panasonic CD-220).

2.5. Ultrastructural preparations

For ultrastructural evaluation by transmission electron microscopy as described previously by Dykstra *et al.* (2002), freshly excised adrenal glands were cut into small blocks ($1\times1mm^3$), fixed directly in cold 4F1G (i.e. 4% formalin + 1% glutaraldehyde adjusted at pH 2.2) for 24 hours, then were post fixed in 1% osmium tetroxide in 0.1M phosphate buffer (pH 7.3), dehydrated in an ethanolic series culminating in 100% acetone, and infiltrated with epoxide resin. After polymerization overnight at 60^oC, semithin sections (0.5 µm) were stained with 1% toluidine blue in 1% sodium borate and examined with light microscope. Areas of cortical cells were selected and the blocks trimmed accordingly. Ultrathin sections (80-90 nm) were cut, mounted on

200 mesh copper grids, and stained with uranyl acetate and lead citrate. The stained grids were examined and photographed by JEOL.JEM-1400-EX-ELECTRON MICROSCOPE at the Central Laboratory of Faculty of Science, Ain Shams University. The photographs were printed on KODABROMIDE F5s GLOSSY Black and White-Schwarzweib- Kodak.

3. Results:

3.1. Histological studies (Haematoxylin and eosin preparations)

3.1.1. Control adrenal cortex

Figure (1) showing the three zones of adrenal cortex; zona glomerulosa, fasciculata and reticularis, respectively. As shown in figure (2), the adrenal gland is surrounded by a fibrous connective tissue capsule. Zona glomerulosa is formed of columnar or rather pyramidal cells arranged in glomeruli-like structure, which are separated by delicate trabeculae extending from the capsule. Its cells contain acidophilic cytoplasm with fairly large rounded to oval basophilic nuclei having distinct nucleoli (Fig. 2).

Zona fasciculata is composed of polyhedral or columnar cells arranged in one or two cell thick in long radial cords or fasciculae and they are separated by narrowed blood capillaries lined with endothelial cells. The cells have granulated eosinophilic cytoplasm embodying spherical basophilic nuclei showing distinct nucleoli. Binucleate cells are seen frequently (Fig. 3).

Zona reticularis is characterized by an irregular anastomosing network of intermingled cords separated by numerous wide blood sinusoids lined with endothelial cells. The cells of these cords are columnar cells having moderately eosinophilic cytoplasm, containing certain discrete granules and have rounded basophilic nuclei possessing centrally located nucleoli (Fig. 4).

3.1.2. Ketoconazole-treated adrenal cortex

Generally, the adrenal gland showed enlargement in size with its outer cortex showing hypertrophy as seen in figure (5). The fibrous connective tissue capsule being thickened with increased fibrous elements (Fig. 6).

Glomerulosa, fasciculata and reticularis cells exhibiting hypertrophy with accumulated variable sized lipid droplets in their cytoplasm. Some of these lipid droplets fused together and occupied almost the entire cytoplasm, thus displacing the nuclei eccentrically which showing clear signs of pyknosis, karyorrhexis and karyolysis as clearly observed in figures (6-8).

3.2. Ultrastructural Studies

3.2.1. Control adrenal cortex

Fine structure of zona glomerulosa cells reveal different mitochondrial configuration varying from oval to spherical shapes with a specific tubulo-saccular cristae. In addition, a fair amount of smooth endoplasmic reticulum, small Golgi vesicles and abundant number of lipid droplets are evident. The nuclei of these cells are rounded or oval in shape; sometimes wavy in appearance ensheathed by double nuclear envelopes and possessing nucleoli, peripheral dense heterochromatin and homogenous euchromatin material (Figs. 9-11).

Figures (12-14) exhibit the fine characteristic features of fasciculata cells including; abundance of rounded mitochondria with obvious tubular cristae, smooth endoplasmic reticulum in the form of branching tubules, scanty rough endoplasmic reticulum, fair amount of lysosomes and richness of lipid droplets. The nuclei are large, rounded, possessing prominent nucleoli, dense peripheral heterochromatin, lightly stained euchromatin and surrounded by double nuclear membranes. Blood capillaries lined with endothelial cells are noticed between these fasciculata cells (Fig. 12).

Zona reticularis cells are distinguished by their richness of rounded mitochondria with intensely tubular cristae, smooth endoplasmic reticulum, lysosomes and lipid droplets with varying sizes. Their nuclei are spherical or ovoid in shape contained condensed heterochromatin, euchromatin and prominent nucleoli (Figs. 15-17). Widened and clear blood sinusoids lined with endothelial cells are manifested in figure (15).

3.2.2. Ketoconazole- treated adrenal cortex

Marked ultrastructural changes of zona glomerulosa cells are illustrated in figures (18-20); their cytoplasm contain hypertrophied mitochondria with more electron dense matrices and some of them possessing small vacuolar degenerations, in addition to lysosomes and lipid droplets of variable sizes. The nuclei being electron dens, showing shrinkage, and signs of pyknosis. They are surrounded by irregular nuclear envelopes and containing electron dense nucleoli, heterochromatin and euchromatin.

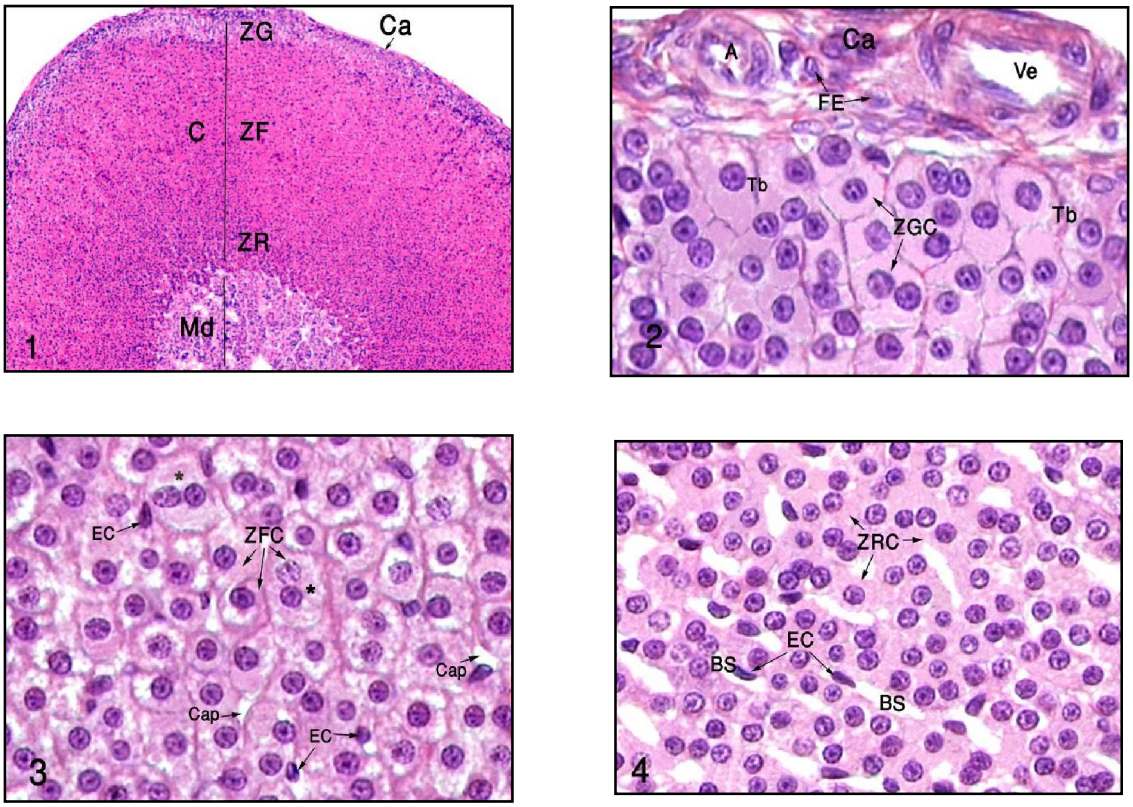
Zona fasciculata cells showing hypertrophied mitochondria filled with tightly packed tubular cristae, some of them having cavitaion and finally these mitochondria are identified only by the remainder cristae adjacent to the inner boundaries of the limiting membrane. Extensive accumulation of various sized lipid droplets, some of them became so large thus occupying almost the entire cytoplasm, masking the organelles and distending the cells. Fair amounts of lysosomes are seen, beside, the nuclei which displayed signs of pyknosis and karyolysis. Some

blood cells are shown in between fasciculata cells (Figs. 21-23).

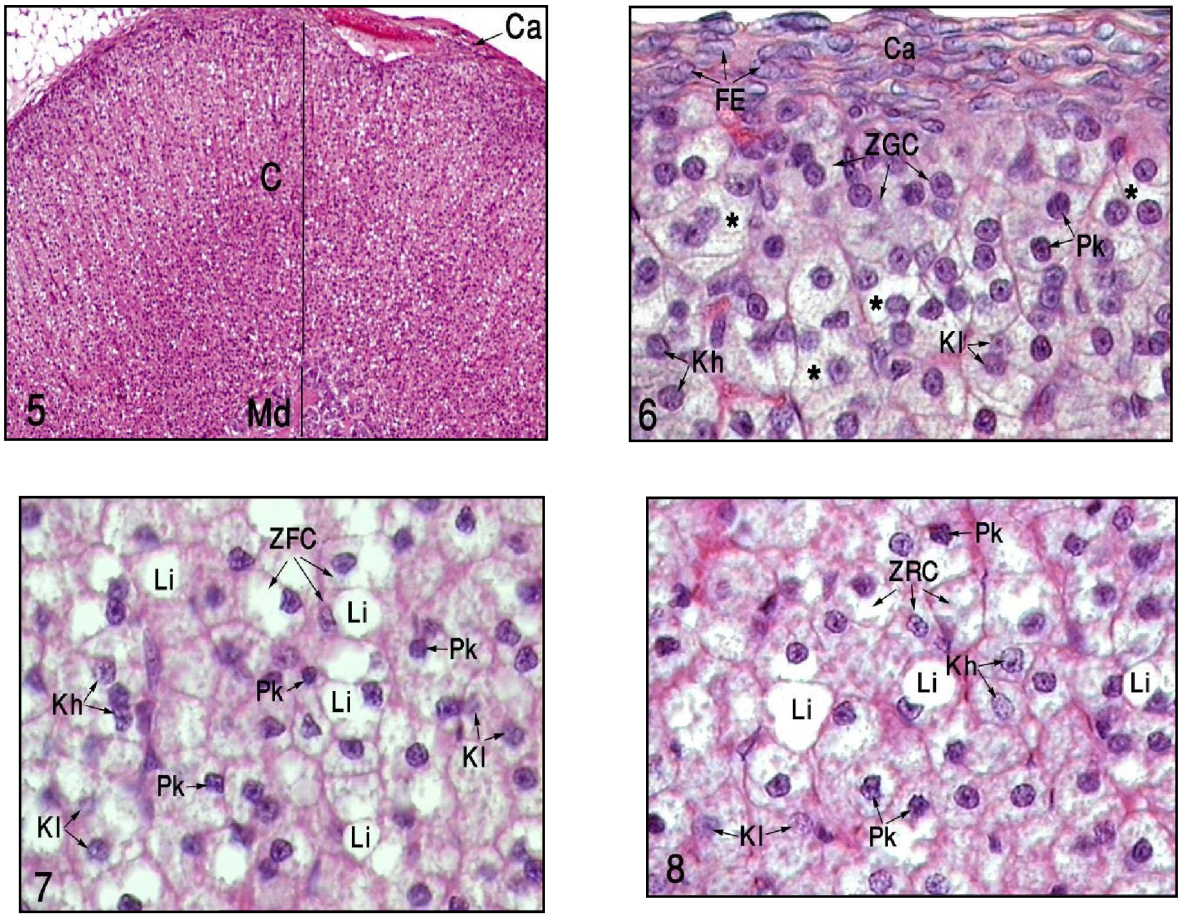
Similarly, zona reticularis cells having hypertrophied mitochondria with some of them showing ruptured mitochondrial membrane, degenerated cristae and cavitation, in addition to accumulated lipid droplets of different sizes, and pyknotic nuclei surrounded by irregular nuclear membrane and containing electron dense heterochromatin and euchromatin. As well as, blood sinusoids containing stagnant blood cells are observed (Figs. 24-26).

It is worthy to mention that smooth endoplasmic reticulum, was scanty, sometimes almost absent in all examined cells of the adrenocortical zones, suggesting that it may be disintegrated under the influence of ketoconazole.

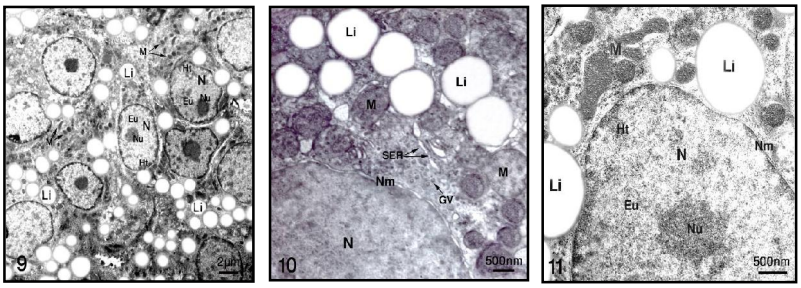
An interesting observation is seen in the resulted electron micrographs, that there are dense particles participated allover the cells of these three zones, which may have occurred as a result of a chemical reaction between ketoconazole and the chemical components of the cells.



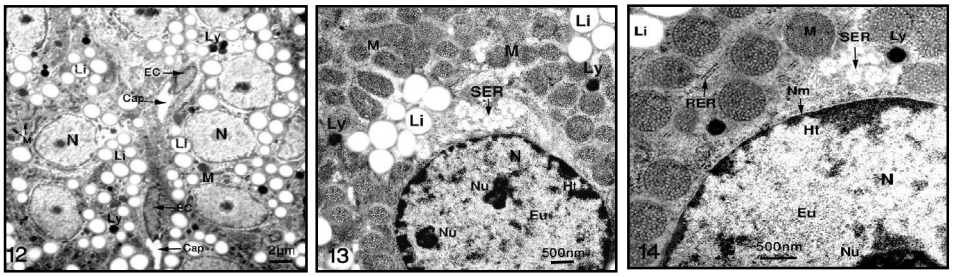
Figures 1-4: Light micrographs of H&E stained sections of control adrenal gland.
Figure 1: General structure of adrenal gland illustrating the capsule (Ca), the cortex (C) which is differentiated into zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR), and the medulla (Md) (x132).
Figure 2: Glomerular organization of zona glomerulosa cells (ZGC), being separated by trabeculae (Tb) extended from the capsule (Ca), which is formed of fibrous elements (FE), an arteriole (A) and a veinule (Ve) (x1320).
Figure 3: Zona fasciculata cells (ZFC) arranged in long radial cords, separated by narrowed blood capillaries (Cap) lined with endothelial cells (EC). Binucleate cells (*) are also seen (x1320).
Figure 4: Zona reticularis cells (ZRC) arranged in irregular network of intermingled cords, separated by numerous wide blood sinusoids (BS) lined with endothelial cells (EC) (x1320).



Figures 5-8 Light micrographs of H&E stained sections of ketoconazole-treated adrenal gland.
Figure 5: Adrenal gland with an enlarged outer cortex (C), an inner medulla (Md) and a thick fibrous capsule (Ca) (x132).
Figure 6: Hypertrophied glomerulosa cells (ZGC), containing lipid droplets (*) and their nuclei showing signs of pyknosis (Pk), karyorrhexis (Kh) and karyolysis (Ki), in addition to part of thickened capsule (Ca) with increased fibrous elements (FE) (x1320).
Figure 7: Hypertrophied fasciculata cells (ZFC), overloaded with lipid droplets (Li) of variable size and possessing necrotic nuclei revealing signs of pyknosis (Pk), karyorrhexis (Kh) and karyolysis (Kl) (x1320).
Figure 8: Enlarged reticularis cells (ZRC) containing lipid droplets (Li) with different sizes and necrotic nuclei revealing pyknosis (Pk), karyorrhexis (Kh) and karyolysis (Kl) (x1320).



Figures 9-11: Transmission electron micrographs of control zona glomerulosa.
Figure 9: Zona glomerulosa cells displaying numerous lipid droplets (Li), mitochondria (M) and oval or round shaped nuclei (N) (x2000).
Figure 10: Glomerulosa cell having mitochondria (M) with tubulo-saccular cristae, smooth endoplasmic reticulum (SER), small Golgi vesicles (GV), lipid droplets (Li) and part of the nucleus (N) ensheathed by a double nuclear envelope (x10,000).
Figure 11: Another glomerulosa cell possessing mitochondria (M) with tubulo-saccular cristae, lipid droplets (Li) and part of the nucleus (N) ensheathed by a double nuclear envelope and involving nucleolus (Nu), marginated dense clumps of heterochromatin (Ht) and homogenous euchromatin (Eu) (x12,000).

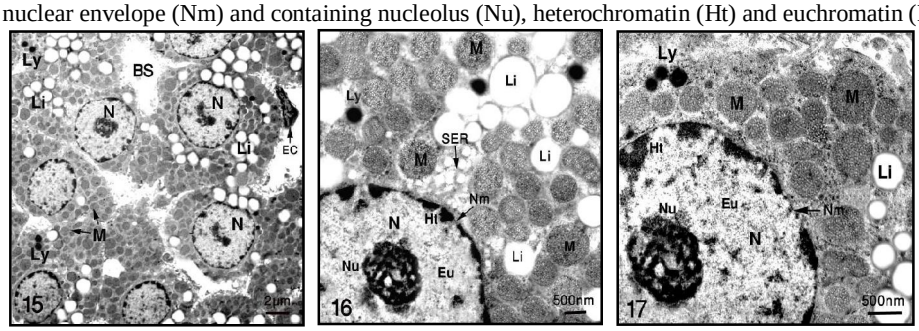


Figures 12-14: Transmission electron micrographs of control zona fasciculata.

Figure 12: Zona fasciculata cells loaded with lipid droplets (Li), mitochondria (M), lysosomes (Ly), and possessing oval to rounded nuclei (N). In between them, narrowed blood capillaries (Cap) lined with endothelial cells (EC) are noticed (x2000).

Figure 13: Fasciculata cell containing mitochondria (M) with tubular cristae, smooth endoplasmic reticulum (SER), lysosomes (Ly), lipid droplets (Li), rounded nucleus (N) containing nucleoli (Nu), peripheral heterochromatin (Ht) and euchromatin (Eu) (x10,000).

Figure 14: Another part of fasciculata cell revealing rounded mitochondria (M) with tubular cristae, smooth endoplasmic reticulum (SER), rough endoplasmic reticulum (RER), lysosomes (Ly), lipid droplets (Li), nucleus (N) surrounded by double nuclear envelope (Nm) and containing nucleolus (Nu), heterochromatin (Ht) and euchromatin (Eu) (x12,000).

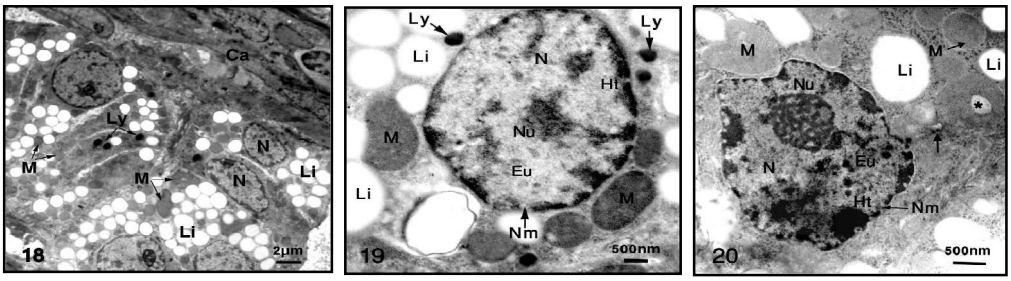


Figures 15-17: Transmission electron micrographs of control zona reticularis.

Figure 15: Reticularis cells containing numerous mitochondria (M), lipid droplets (Li) and spherical or ovoid nuclei (N). Widened blood sinusoids (BS) lined with endothelial cell (EC) are obviously seen (x2000).

Figure 16: Reticularis cell having mitochondria (M), smooth endoplasmic reticulum (SER), lysosomes (Ly), lipid droplets (Li) and nucleus (N) which is surrounded by nuclear membrane (Nm) and possessing distinct nucleolus (Nu), peripheral heterochromatin (Ht) and euchromatin (Eu) (x10,000).

Figure 17: Another reticularis cell possessing nucleus (N) containing distinct nucleolus (Nu), peripheral heterochromatin (Ht) and euchromatin (Eu) and is surrounded by nuclear envelope (Nm), in addition to numerous rounded mitochondria (M), aggregation of three lysosomes (Ly) and lipid droplets (Li) (x12,000).

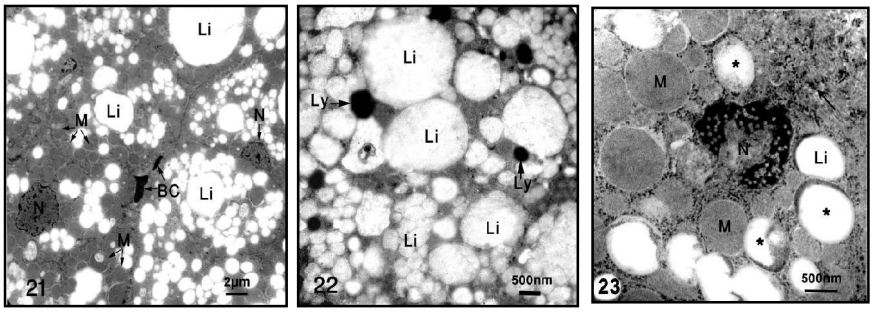


Figures 18-20: Transmission electron micrographs of ketoconazole-treated zona glomerulosa.

Figure 18: Zona glomerulosa cells having mitochondria (M), lipid droplets (Li), lysosomes (Ly) and deformed nuclei (N), in addition to deformed capsule (Ca) (x2000).

Figure 19: Glomerulosa cell illustrating electron dense hypertrophied mitochondria (M), lysosomes (Ly), lipid droplets (Li) and nucleus (N) surrounded by nuclear membrane (Nm) and containing electron dense peripheral heterochromatin (Ht) and euchromatin (Eu) (x10,000).

Figure 20: Another glomerulosa cell exhibiting hypertrophied mitochondria (M), some of them having vacuolar degeneration (*), lipid droplets (Li), dense particles participate (arrow→) through the matrix and pyknotic nucleus (N), being shrunken ensheathed by irregular nuclear membrane (Nm) and having electron dense heterochromatin (Ht) and euchromatin (Eu) (12,000).

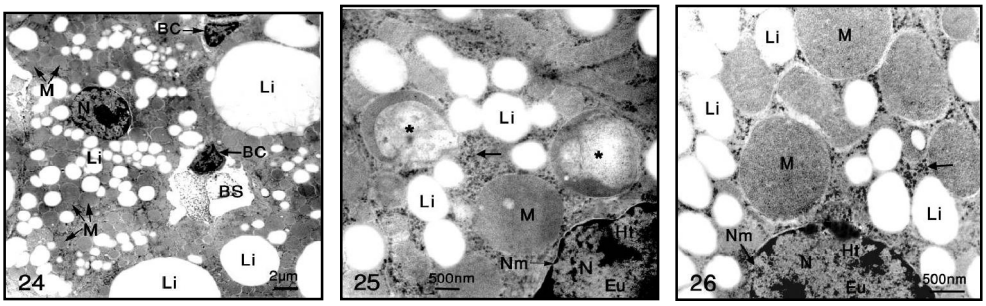


Figures 21-23: Transmission electron micrographs of ketoconazole-treated zona fasciculata.

Figure 21: Zona fasciculata cells overloaded with lipid droplets (Li) of variable sizes, in addition to deformed mitochondria (M) and pyknotic nuclei (N). Blood cell (BC) are seen (x2000).

Figure 22: Fasciculata cell exhibiting progressive accumulation of various sized lipid droplets (Li), in addition to numerous lysosomes (Ly) (x10,000).

Figure 23: Another fasciculata cell revealing hypertrophied mitochondria (M), some of them possessing cavitation with progressive loss of their cristae (*), in addition to lipid droplets (Li), dense particles participate (arrow→) through the matrix and nucleus (N) with signs of karyolysis (X12,000).



Figures 24-26: Transmission electron micrographs of ketoconazole-treated zona reticularis.

Figure 24: Zona reticularis cells having deformed mitochondria (M), lipid droplets (Li) of different sizes and deformed nuclei (N), in addition to blood sinusoids (BS) containing blood cell (BC) (x2000).

Figure 25: Reticularis cell containing hypertrophied mitochondria (M), some of them showing cavitation with ruptured limiting membranes and degenerated cristae (*), in addition to several lipid droplets (Li), dense particles participate (arrow→) through the matrix and part of the nucleus (N) surrounded by nuclear membrane (Nm) and containing electron dense heterochromatin (Ht) and euchromatin (Eu) (x10,000).

Figure 26: Another reticularis cell having extensive accumulation of hypertrophied mitochondria (M) and lipid droplets (Li), in addition to dense particles participate (arrow→) through the matrix and nucleus (N) possessing electron dense heterochromatin (Ht) and euchromatin (Eu) and surrounded by nuclear envelope (Nm) (X12,000).

4. Discussion

There is increasing evidence that various chemicals introduced into the environment have the potential to disrupt the endocrine system in humans and wildlife. Increasingly, the enzymes involved in the steroid biosynthesis pathway are being recognized as important targets for the actions of the endocrine-disrupting chemicals. Steroidogenic enzymes are responsible for the biosynthesis from cholesterol of various steroid hormones including glucocorticoids, mineralcorticoids, androgens, and estrogens. They consist of several specific cytochrome P450 enzymes (CYPs), hydroxysteroid dehydrogenases (HSDs), and steroid reductases (Sanderson, 2006).

In recent years, a number of imidazole derivatives have been developed and used as an anti-fungal agents. Ketoconazole is one of the imidazole derivatives currently used in the clinical fields. It is an effective inhibitor of adrenal and gonadal steroidogenesis (Schimmer and Parker, 2006). For this property, it has been used for the treatment of different types of diseases including; Cushing disease, hyper-cortisolemic depressive patients, adrenal tumors, adrenocortical carcinoma, adrenal adenomas, prostate cancer, advanced breast cancer and various cancer cell lines such as hepatic metastasis and pulmonary metastasis (Lionakis *et al.*, 2008).

The majority of severe degenerative changes induced in the adrenal gland following pathological disorders are often judged by the physiological tools. Steroidogenesis inhibition of ketoconazole has also been studied in experimental animals by O`Connor

et al., (2002), which evaluated a 15-days screening assay using intact male rats for identifying physiologically steroid biosynthesis inhibition of ketoconazole, also Shin *et al.* (2006) elucidated a 28-days repeated dose toxicity study of ketoconazole in rats. These authors reported in their interesting researches that ketoconazole caused increase of adrenal's weights, reflecting on the level of these hormones; decrease of testosterone, increase of estradiol, luteinizing hormone (LH) and follicular stimulating hormone (FSH). They suggested that ketoconazole should be identified as an impairment of endocrine-related compound.

The adrenal gland is the most important steroidogenic tissue in the human body and essential for survival. All steroidogenic processes take place in the adrenal cortex (Bielohuby *et al.*, 2007). The adrenal cortex, and in particular zona fasciculata has been reported by Rosol *et al.* (2001) to be among the most common site lesions in the endocrine system. The factors which predispose this organ to such lesions include: its disproportionately large blood supply per unit mass; its high content of lipids and the susceptibility of its unsaturated fatty acids to peroxidation damage; and its high levels of cytochrome P450 which metabolize xenobiotics to reactive intermediates. In addition, the adrenal expresses several of the pathways for steroid production present in the testes and ovaries. Therefore, toxic chemicals can affect the adrenal or its axis directly or indirectly in a manner similar to the testes and ovaries.

The present study throws the light on the impact of ketoconazole on adrenal cortex from the histological and ultrastructural point of view, because these types of studies did not receive marked attention in spite of its importance to characterize lesions that may suppress the function of the adrenocortical cells.

In the present investigation, ketoconazole is found to have destructive structural and ultrastructural alterations in rat adrenal cortex. The most striking change is the enlargement and extensive accumulation of lipid droplets throughout the cytoplasm of the three zones cells. This alteration was also reported in the influence of chronic treatment with aminoglutethimide, an inhibitor of the cholesterol enzyme $P450_{SCC}$ (Szabo *et al.*, 1996), congenital lipoid adrenal hyperplasia in humans and mice, a disorder caused by hereditary deficiency of the StAR protein (Miller and Strauss, 1999), hormone-sensitive lipase (HSL) deficiency in mice (Li *et al.*, 2002) and after chemotherapy administration (Hermenean *et al.*, 2008).

In this work, distinct alterations are evidenced in the mitochondria displayed by hypertrophy with degeneration of the cristae resulting in cavitation of their matrix. These lesions are not reported previously in rat adrenal cortex. In our opinion this hypertrophy and cavitations in mitochondria probably resulted from inhibition of the conversion of cholesterol to pregnenolone, cholesterol may accumulate within the mitochondria. Consequently, it undergo considerable hypertrophy and vacuolation. Also, similar results were observed by Ishihara *et al.* (1974) in human adrenal cortex in Cushing's syndrome. They explained that the intra-mitochondrial vacuoles might be associated with the deposition of steroids or their related compounds in the cristae.

The profound lesions observed in mitochondria and smooth endoplasmic reticulum might be sufficient to cause impairment of steroid synthesis in accordance with Guerrero *et al.* (2010), who reported that these organelles play a great role in steroidogenesis within the cortex, though, they involve in the coordinated actions of cytochrome P450 and the enzyme 3β-hydroxysteroid dehydrogenase (3βHSD), which are distributed between the mitochondria and the smooth endoplasmic reticulum. The rate-limiting step in steroid hormone biosynthesis is the translocation of substrate cholesterol from the outer mitochondrial membrane to cholesterol side-chain cleavage enzyme (CYP11A), the first enzyme in the steroidogenic pathway, which is located inside the mitochondria as Rainey *et al.* (2004) and Isola *et al.* (2010) elucidated in their interesting studies.

It is well known that, adrenocortical cells require constant supply of cholesterol as a precursor for the conversion of steroid hormones. Cholesterol delivery in the adrenal glands involves three major processes: uptake of lipoprotein-derived cholesterol via low density lipoprotein receptor (LDLR) mediated endocytic pathways and scavenger receptor class B member 1 (SCARB1)-mediated "selective" uptake pathways; endogenous cholesterol biosynthesis in endoplasmic reticulum; and cholesterol mobilization from intracellular cholesterol esters (CEs) stored in lipid droplets (Kraemer, 2007). The delivered CEs should be hydrolyzed to be utilized for steroidogenesis by non-lysosomal neutral lipases. Therefore, CE hydrolysis plays a pivotal role not only in the break down of stored lipids but also in the lipoprotein uptake and utilization. The resultant unesterified cholesterol is transported to mitochondria by the steroidogenic acute regulatory protein (StAR), where it is converted into the different steroid hormones by a battery of oxidative enzymes (Miller, 2007).

Ketoconazole was found to inhibit cholesterol synthesis in a dose-dependent fashion by blocking

conversion of lanosterol to cholesterol. Other lipid-modifying properties of ketoconazole include decreasing of lipoprotein lipase and 3-hydroxy-3-methylglutaryl coenzyme A reductase activities, inhibition of intestinal cholesterol absorption and bile acid synthesis, and upregulation of LDL-C receptor activity (Lionakis *et al.*, 2008).

Ultimately, It seems, that the impaired steroidogenesis is an important mechanism of toxicity in the adrenal cortex. It may have occurred due to disruption of cytochrome P450 enzymes, accordingly the cholesterol biosynthesis will be suppressed. This will lead to the accumulation of lipid droplets that seen in the electron micrographs, indicating that the target organ of ketoconazole on adrenocortical cells are mitochondria since the treated animals had destructive mitochondria on which cytochrome P450 enzymes are enclosed.

In conclusion, it is noticed now that the destructive impacts of ketoconazole on the adrenocortical cells reflected on their functions leading to much deficiency in their performance. So, it should be taken in consideration and great concern that this drug must be utilized under restricted precautions in the medical fields to protect the human health from its hazardous impact.

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Inflammatory Allergic Immune Response in Scabies Pyoderma

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Abstract: Scabies is a highly pruritic disorder induced by an immune allergic response to infestation of the skin by the mite sarcoptes scabiei that burrows in the stratum corneum of the skin. Scabies persists despite the availability of topical insecticides because the disease is transmitted before it is diagnosed and treated and significant resistance to insecticides has developed. The aim of this study was to investigate immunobiochemical response of the host's inflammatory/immune response to scabies during infestation in schoolchildren at rural areas. The number of eosinophil, neutrophil, serum IgE and serum interleukin (IL)-5 were assessed in 21 patients with 20 healthy control subjects. In the present study significantly higher number of eosinophil (11.90 ± 2.0 %vs. 4.12 ± 1.75 %; p<0.001) and highly significant elevation in neutrophil (%) in the scabies group compared to the healthy controls (39.9 ± 5.2%vs. 23.5 ± 2.0%, p<0.001). There was a highly significant elevation in serum IgE in the scabies group compared to the healthy normal controls (150 ± 58 IU/ml vs. 36.7 ± 18.0 U/ml p<0.001). As regards serum IL-5, there was a highly significant elevation in Interleukin-5 in the scabies group compared to the healthy normal controls (44.0 ± 15.3 ug/l vs. 12 ± 3.6 ug/l p<0.001).The results of this study suggest that there is a potent inflammatory immune response during the infestation of sarcopties scabiei. In conclusion, scabies is a dangerous disease affecting the immune system and should be treated once discovered to prevent the hazards of being epidemics.

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Key Words: Scabies; Eosinophil; IgEl; Interleukin (IL)-5; risk factors of scabies; epidemics.

1. Introduction

Scabies is a common parasitic infestation of global proportion. Worldwide, an estimated 300 million cases occur annually [15]. The arthropod *Sarcoptes scabiei* var *hominis* causes an intensely pruritic and highly contagious skin infestation which affects males and females of all socioeconomic status and ethnic groups [14]. Scabies infestation has been reported for more than 2500 years. Aristotle discussed "lice in the flesh," which resulted in vesicles, and Celsus recommended sulfur mixed with liquid pitch as a remedy for the disease [10]. However, the disease was first ascribed to the mite by Giovan Cosimo Bonomo in 1687. It was the first human disease recognized to be caused by a specific pathogen.

Mode of Transmission

Transmission of scabies is predominantly through direct skin-to-skin contact, and for this reason, scabies has been considered a sexually transmitted disease. Those at high risk include men who have sex with men and men with sexual contacts [15]. A person infested with mites can spread scabies even if he or she is asymptomatic. There may be a prolonged interval (up to 10 wk) between the primary infestation, when the patient becomes contagious, and the onset of clinical manifestations. It is less frequently transmitted by indirect contact through fomites such as infested bedding or clothing. However, the greater the number of parasites on a person, as in crusted scabies, the more likely that indirect contact will transmit the disease [14].

The *S scabiei* var *hominis* mite that infests humans is female and can be seen with the naked eye (0.3-0.4 mm long). The male is about half this size. The mite has 4 pairs of legs. It does not penetrate deeper than the outer layer of the epidermis (Figure 1). Mites are unable to fly or jump. They crawl at a rate of 2.5 cm/min. While the mite's life cycle occurs completely on its host, they are able to live on bedding, clothes, or other surfaces at room temperature for 2-3 days, while remaining capable of infestation and burrowing. At temperatures below 20°C, *S scabiei* are immobile, although they can survive such temperatures for extended periods [16].

Life cycle:

The scabies mite is an obligate parasite and completes its entire life cycle on humans. Other variants of the scabies mite can cause infestation in other mammals such as dogs, cats, pigs, and horses, and these variants can irritate human skin as well. However, they are unable to reproduce in humans and only cause a transient dermatitis. Eggs incubate and hatch in 3-4 days (90% of the hatched mites die).

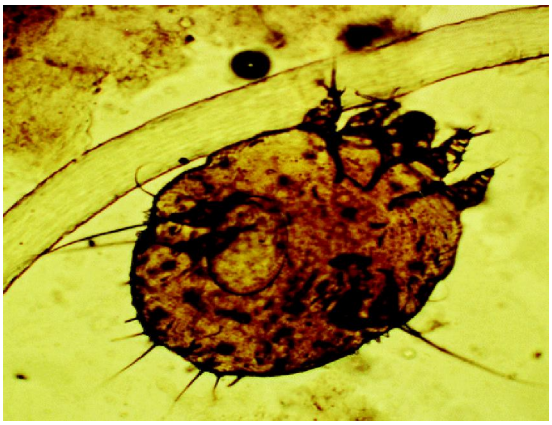

FIG. 1. Female scabies mite with egg, taken from skin scraping [16].

Larvae (3 pairs of legs) migrate to the skin surface and burrow into the intact stratum corneum to make short burrows, called molting pouches (3-4 d). Larvae molt into larger nymphs and then into adults. Mating takes place once, and the female is fertile for the rest of her life (1-2 mo), and the male dies soon after mating. She makes a serpentine burrow using proteolytic enzymes to dissolve the stratum corneum of the epidermis, laying eggs in the process, and she continues to lengthen her burrow and lay eggs for the rest of her life (1-2 mo). Transmission of impregnated females from person-to-person through direct or indirect skin contact. The time required to induce immunity in primary infestations probably accounts for the latent period of 4 weeks of asymptomatic infestation. In reinfestation, the sensitized individual may develop a rapid reaction (within hours). The resultant skin eruption, and its associated intense pruritus, is the hallmark of classic scabies [14].

Frequency:

International

While many accounts of the epidemiology of scabies suggest that epidemics or pandemics occur in 30-year cycles, this may be an oversimplification of its incidence. These accounts coincided with the major wars of the 20th century. Because it is not a reportable disease, and data are based on variable notification, the incidence of scabies is difficult to ascertain. Although epidemics have been reported (1919-1925, 1936-1949, 1964-1979), it is clearly an endemic disease in many tropical and subtropical regions. Scabies is 1 of the 6 major epidermal parasitic skin diseases (EPSD) that is prevalent in resource-poor populations. Prevalence rates are extremely high in aboriginal tribes in Australia, Africa, South America [10], and other developing regions of the world. Incidence in parts of Central America and South America and in one Indian village approach 100%. In parts of Bangladesh, the number of children with "the itch" exceeds the number with diarrheal and respiratory diseases combined [14]. In 2009 retrospective study of 30,078 children in India, scabies was found to be the second most common skin disease in all age groups of children, and the third most common skin disease in infants [4].

Worldwide, the prevalence of scabies has been estimated at 300 million cases annually [5]. In the United States and in other developed regions around the world, scabies occurs in epidemics in nursing homes, hospitals, long-term care facilities, and other institutions. It is seen frequently in the homeless populations but occurs episodically in other populations as well. No recent published data are available on its incidence in the United States. Studies conducted in Brazil [6&7] identified major risk factors for scabies in an impoverished rural community. The risk factors were young age, presence of many children in the household, illiteracy, low family income, poor housing, sharing clothes, and towels, and irregular use of showers.

Mortality/Morbidity:

Hundreds to millions of mites infest the host individual, who is usually immunocompromised, child, elderly, or physically or mentally disabled and impaired. Crusted scabies can be easily confused with severe dermatitis or psoriasis because widespread, crusted lesions appear with thick, hyperkeratotic scales over the elbows, knees, palms, and soles. The diagnosis of crusted scabies should be considered when suspected dermatitis or suspected psoriasis do not respond to usual treatments. Serum immunoglobulin E (IgE) IgG immunoglobulins, and neutrophil levels are extremely high in these patients, yet the immune reaction does not seem to be protective. Cell-mediated immunity in classic scabies demonstrates a predominantly CD4 T-cell infiltrate in the skin [10 & 16]. It has been suggested that bullous scabies could develop after long persistency of the parasites leading to a specific immune response with activation of T-helper type-2 cells leading to high levels of the cytokine interleukin 5 and subsequent eosinophilia. Secretion of proteolytic enzymes near the basal membrane zone might explain the development of intra-epidermal, often suprabasal blisters. In a patient with a positive biopsy and immunofluorescence result, the scabies infestation may have triggered a flare of the underlying autoimmune disease [3].

As a result, it is hypothesized that scabies

may exist in a great population of rural Beni-Suef, Egypt. The aim of this study was to discover and measure the inflammatory markers in infested patients compared to controlled group hoping a new future for a neglected disease to be treated.

2. Subjects and Methods

The consent of the parents of schoolchildren in rural areas of Beni-Suef, Egypt was taken. The schools of these children were built inside the village near the animals. History taking especially itching and scratching, more than the surface of the skin, and good questionnaire with thorough examination of skin were done. Twenty-one patients with age range 6-7 years of age with totally irritated skin due to scabies infestation (crusted type) were selected from whom were compaining severe itching. We have known about their low socio-economical conditions, big family size, malnutriton, and bad hygiene in dirty culture (we searched about skin rash at hand, between fingers, wrist, elbow, lower abdominal, genital or scrotum with severe itching specially at night, infected skin regions- from scratching).

The diagnosis of scabies is relatively easy for the physicians. A physical examination revealed the tell tale characteristics of scabies, and a skin scraping from a burrow can reveal the presence of the actual mite, although in most cases the skin scraping isn't necessary. To confirm diagnosis [13] superficial scraping was done and examination, under a low-power microscope, of material that has been expressed from a burrow revealed the mite, ova, or mite feces by the entomology specialist. The diagnosed subjects (21 out of 34) were compared with 20 control subjects from urban areas with completely normal skin.

All subjects were asked about the asthmatic symptoms and the requirement of rescue medication to be excluded. Analysis of both stool and urine was done also to exclude the other parasitic causes.

By using Sysmex cell counter, we took a blood sample to measure:

The white blood corpuscle count including both the eosinophil and neutrophil.

Eosinophil count was performed using Wright's stain [12]. (a specially prepared mixture of methylene blue and eosin in methanol, used in staining blood smears). Five hundred non-squamous cells were counted in Wright's-stained slides and the results were expressed as percentage of the non-squamous counts.

- Serum immunoglobulin IgE was measured (IU/ml) by immuno-nephelometric assay; Dade Behring Marburg GmbH/ Germany
- Interleukin (IL)-5 was measured (µg/ml) by quantitative 'sandwich' enzyme-linked immunosorbent assay using IL-5 kit (Sigma chemical Co., St. Louis, USA) according to the manufacturer's instructions

Prevention and Control of scabies

Human scabies is caused by an infestation of the skin by the human itch mite (Sarcoptes scabiei var. hominis). The microscopic scabies mite burrows into the upper layer of the skin where it lives and lays its eggs. The most common symptoms of scabies are intense itching and a pimple-like skin rash. The scabies mite usually is spread by direct, prolonged, skin-to-skin contact with a person who has scabies. [1].When a person is infested with scabies mites the first time, symptoms may not appear for up to two months after being infested. However, an infested person can transmit scabies, even if they do not have symptoms. Scabies usually is passed by direct, prolonged skin-to-skin contact with an infested person. However, a person with crusted (Norwegian) scabies can spread the infestation by brief skin-to-skin contact or by exposure to bedding, clothing, or even furniture that he/she has used. [11, 4].

Scabies is prevented by avoiding direct skin-to-skin contact with an infested person or with items such as clothing or bedding used by an infested person. Scabies treatment usually is recommended for members of the same household, particularly for those who have had prolonged skin-to-skin contact. All household members and other potentially exposed persons should be treated at the same time as the infested person to prevent possible reexposure and reinfestation. Bedding and clothing worn or used next to the skin anytime during the 3 days before treatment should be machine washed and dried using the hot water and hot dryer cycles or be dry-cleaned. Items that cannot be dry-cleaned or laundered can be disinfested by storing in a closed plastic bag for several days to a week. Scabies mites generally do not survive more than 2 to 3 days away from human skin. Children and adults usually can return to child care, school, or work the day after treatment. . [4].

A scabies prevention program involves:

The infection control practitioner should be responsible for (1) identification of contacts of symptomatic case(s), (2) prevention of transmission, (3) treatment of symptomatic cases, (4) treatment of contacts, (5) post-treatment assessment and (6) assessment of treatment failures. [4]. All levels of the health care team. The program should include an assessment of the skin, hair and nail beds of all new admissions as soon as possible following arrival. Pruritus, rashes and skin lesions should be documented and brought to the attention of the

nursing supervisor and the attending physician for further follow-up.

Essential elements of a successful scabies prevention program include: 1. Written policies and procedures for prevention and control of nosocomial scabies; 2. Health care workers who are trained to be suspicious of scabies in themselves or their patients if unexplained rash or pruritus occurs in themselves or their patients, and to report such occurrences to their supervisors; 3. A policy to screen newly admitted patients for scabies during the initial assessment (especially if transferred from another healthcare facility) and any suspect patient will immediately be placed on contact isolation until examined for scabies; 4. A policy that all new employees (especially employees who work at more than one facility) will be screened for scabies as part of pre-employment screening; 5. Access to and use as needed of the diagnostic skills of a consultant experienced in recognizing scabies to evaluate difficult or unusual cases or response to treatment;6. Assurance of adequate support from hospital administration, medical staff, infection control, employee health and line staff for appropriate evaluation and treatment of employees, in-house patients and exposed discharged patients should an outbreak of nosocomial scabies occur [20].

Statistical Analysis:

Results are expressed as mean ± standard deviation (SD). Data were collected coded and analyzed using SPSS software version 15 under windows XP.

3. Results:

Our study was done on 41 primary schoolchildren from Beni-Suef, Egypt in rural and urban areas, which were divided into two groups: Scabies group (9M/12F) and the healthy control group (10M/10F), both were matched in age and sex. Scabies group was proved to be of low socioeconomical conditions and ignore good hygienic measures in opposite to the control group as shown in table 1.

Table 1. Subjects' Clinical Characteristics

	Scabies group (n = 21) From rural areas	**Healthy control group (n = 20) From urban areas**
Age (years)	6 ± 0.7	6 ±.0.5
Sex,(male : female)	9 : 12	10 : 10
Socioeconomical conditions (Nutrition)	Bad	Good
Hygienic measures	Bad	Good
Skin morphology	Deep scratches	Normal intact skin

Table 2: Descriptive statistics of the different studied variables in the studied groups

Variables	**Scabies group (n = 21)**	**Healthy control group (n =20)**	***P* value**
Eosinophil (%)	11.90 ± 2.0	4.12 ± 1.75	0.001
Neutrophil (%)	39.9 ± 5.2%	23.5 ± 2.0	0.001
Serum IgE (IU/ml)	150 ± 58	36.7 ± 18.0	0.001
Interleukin-5 (μg/l)	44.0 ± 15.3	12 ± 3.6	0.001

P<0.001 is highly significant

As table 2 illustrates, there was a highly significant elevation in Eosinophil (%) in the scabies group compared to the healthy controls (11.90 ± 2.0 %vs. 4.12 ± 1.75 %, p<0.001). There was also a highly significant elevation in Neutrophil (%) in the scabies group compared to the healthy controls (39.9 ± 5.2%vs. 23.5 ± 2.0%, p<0.001). There was a highly significant elevation in serum IgE in the scabies group compared to the healthy normal controls (150 ± 58 IU/ml vs. 36.7 ± 18.0 U/ml p<0.001). As regardes IL-5, there was a highly significant elevation in Interleukin-5 in the scabies group compared to the healthy controls (44.0 ± 15.3 ug/l vs. 12 ± 3.6 ug/l p<0.001).

4. Discussion:

Scabies is a worldwide disease and a major public health problem in many developing countries, related primarily to poverty and overcrowding. Scabies is more prevalent in children and young adults. Scabies is more common in women than in men, which agreed with our results, and more common in winter than summer [4]. Scabies is very easy to misdiagnose because early subtle cases may look like small pimples or mosquito bites. Over a few weeks, however, mistakes like this become evident as patients feel worse and worse with symptoms they can't ignore.

Scabies is a neglected worldwide health problem. In the U.S., it occurs frequently in the general population and in institutions such as daycare centers and nursing homes. The prevalence is nearly 100% in infants and >50% in older children and women in some populations in the world. Scabies persists despite the availability of topical insecticides because the disease is transmitted before it is diagnosed and treated and significant resistance to insecticides has developed. The first time a person becomes infested with scabies the inflammatory/immune reaction in the skin is delayed for 4-8 weeks and there are no symptoms. The delay in the symptoms suggests that these mites may produce substances that can initially inhibit the host response. Previous studies have shown that mite extracts modulate the cytokine expression of keratinocytes, fibroblasts, monocytes, dendritic cells and lymphocytes. It appears that skin endothelial cells may play a key role in this delay of symptoms. A previous infestation with S. scabiei induces protective immunity. The mechanisms responsible for the delayed primary response and the protective immunity from a previous infestation are not understood [5 &16].

Classic scabies is primarily a nuisance. However, it can indirectly lead to long-term morbidity. The main symptoms of scabies are due to immune reaction to burrowed mites and their products (e.g. faeces, saliva or eggs. Scabies and other parasitic skin diseases can lead to long-term colonization of skin lesions by group A streptococci. Several studies have demonstrated a correlation between poststreptococcal glomerulonephritis (PSGN) and scabies. In remote Aboriginal communities in Australia where scabies is endemic, the repeated infestations and secondary streptococcal infections appear to be related to the extremely high levels of renal failure and rheumatic heart disease observed in the communities. While the microbiology of secondary bacterial infection in scabies lesions probably changes based on geographic location, one study demonstrated that the predominant aerobic and facultative bacteria recovered from lesions were *Staphylococcus aureus,* group A streptococci, and *Pseudomonas aeruginosa.* Multiple anaerobes were recovered as well, suggesting polymicrobial colonization of lesions [9]. Other complications of scabies include impetigo, furunculosis, and cellulitis. The staphylococci or streptococci in the lesions can lead to pyelonephritis, poststreptococcal glomerulonephritis, abscesses, pyogenic pneumonia, sepsis, and death [8]. Chronic inflammation is recognized as the major characteristic of eosinophil seems to play a key role. IL-5 is associated with eosinophilic inflammation. IL-5 is important in the terminal differentiation of eosinophils, in promoting eosinophil survival, and in priming and activating eosinophils [2].

Our patients group showed strong proliferative responses (peripheral blood mononuclear cells) to the scabies antigens, most of patients were crusted scabies which showed increased secretion of the Th2 cytokines interleukin 5 (IL-5). These data confirm that a non-protective allergic response occurs in the crusted disease form and demonstrate that clinical severity is associated with differences in the type and magnitude of the antibody and cellular responses to scabies proteins, in response to the active cysteine protease [17].

The modern concept of personal hygiene not only includes the prevention of diseases by cross-infection, but also the avoidance and removal of dirt which disturb the immune system [1].

Conclusion and recommendations:

Scabies can spread between schoolchildren, especially in rural ares, through close personal contact (relatives, schoolchildren, and crowded communities). Scabies should be treated once discovered to prevent the hazards of being epidemics. The most important factor to limitate the outbreak seems to be the prophylaxis of people who are in contact with the patients like friends, teachers, family members and car-drivers. The most exposed patients seemed to be those with diminished independence, diabetes, post-streptococcal and malnutrition. Consultation with a dermatologist or an infectious disease specialist may be required for severe, refractory scabies or for disseminated scabies in patients who are immunocompromised. Infested children must be treated cauciously with simple prophylactic treatment to close contacts.

The long term goal is to develop a better understanding of the host parasite relationship that will lead to development of novel strategies for the control/prevention and treatment of this disease. Determination of mite secretions and extracts and

testing isolated molecules for immunomodulating activity in several skin and blood cell types should be studied.

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Oriented Indium Doped Zinc Oxide Thin Films by Spray Pyrolysis Technique

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Abstract: Spray pyrolysis technique has been proved to be an effective tool to produce well oriented undoped and indium doped zinc oxide thin films onto glass substrates. The correlation between the main preparation parameters such as: substrate temperature (673-873 K), spraying time (10-50 min.) and doping concentration (0-3%) and the structural and optical properties of the films, have been discussed. The alignment of the crystals has been investigated by X-ray diffraction, atomic force microscopy and transmission electron microscopy. The prepared ZnO:In films are generally homogeneous, have smooth surfaces and low sheet resistance values R_s in kΩ range. The doping with Indium decreased the sheet resistance of ZnO films by about two orders of magnitude.

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Keywords: Zinc oxide, Indium, Spray Pyrolysis, Mono-crystals, Single-crystals.

1. Introduction

Zinc oxide (ZnO) is a good candidate for different opto-electronic devices; gas sensors, ultrasonic oscillators and transducers. Additionally, in solar cell related applications, for instance, as a window material, or in a heterojunction solar cell ZnO/CdTe, ZnO can be used due to its mix of properties like stability in hydrogenated atmosphere and non-toxicity [1-5]. ZnO films have been prepared by different techniques such as: sputtering [6], chemical vapor deposition [7], and spray pyrolysis technique [8-10]. Spray pyrolysis may have several advantages compared with the other techniques such as: simplicity and possibility to produce non-expensive large area films.

In this paper, it was attempted to establish an idea that spray pyrolysis technique could be an effective tool to prepare well-oriented mono crystals. That is why the structural characteristics of undoped and Indium doped ZnO films prepared by spray pyrolysis are investigated.

2. Experimental

The ZnO:In films were deposited on glass substrates with dimensions 11x22 mm (Menzel-Gläser, Germany) by the spray pyrolysis set up which had been already described in an earlier publication [11]. Zinc acetate (Merck, 99%) was dissolved in a mixture of three quarters water (HPLC grade) and one-quarter methanol (Laborchemie Apolda, p.a., Germany). The deposition parameters were: 0.2 molarity of the starting solution, 1.1 bar air pressure, 0.3 ml/min solution rate, (673-873 K) substrate temperature range and (10-50 min.) spray time range. The doping is achieved by adding Indium trichloride to the solution with percentages 1-3% and at the same Zn molarity (0.2 M).

X-ray diffractometery (SIEMENS D5000) and transmission electron microscopy TEM in conjunction with energy dispersive X-ray spectroscopy EDX were used to get a more detailed view of the microstructure and composition of the prepared films. For TEM imaging, a HITACHI H-8100 II electron microscope was used at an operation voltage of 200 kV (point to point resolution 0.23 nm), with attached EDX detector (Oxford Instruments, Link ISIS). Atomic force microscope AFM (JENAVAL, Carl Zeiss, equipped with a SIS-Ultra objective) was used to get information about the surface topography of the films. The UV/VIS/NIR-transmission spectra of the samples were recorded by a spectrophotometer (UV-3101PC Shimadzu, Japan) to investigate the optical properties of the prepared films.

3. Results

X-ray diffraction pattern XRD of undoped and 1% Indium doped ZnO films at two substrate temperatures T_s, namely 773 & 873 K, at the same deposition time t_d = 30 min., besides the ZnO JCPDS data file 36-1451 for comparison, are shown in Fig.1, which indicates that the films are polycrystalline and well oriented in [002] direction of the hexagonal Wurzite ZnO.

A TEM image of ZnO: 1%In film prepared at substrate temperature T_s = 873 K and deposition time t_d = 30 min. is presented in Fig. 2, which shows well oriented large crystals.

Fig.3 shows the transmission T in percent, against the wavelength λ in nm, at different substrate temperatures T_s and at the same deposition time t_d = 30 min. for undoped and 2% Indium doped ZnO films. The films are transparent (≈ 70-90%) in the visible region.

The T - λ curve of undoped and indium doped ZnO at substrate temperature T_s = 873 K, deposition time t_d = 30 min. and different doping concentrations, can be seen in Fig.4, the doping with Indium (1-3%)

lower the transparency of ZnO films from almost 90% to 70%. The thickness of the films and related optical constants have been estimated using a software package (APAS Program, Ver.1.3, J.K. Kühn, Otto-Schott-Institut, Germany) on the basis of experimental optical transmission spectra.

The atomic force microscopic image AFM of ZnO: 1%In, at substrate temperature T_s = 873 K and deposition time t_d = 30 min. is shown in Fig.5, which shows the topography and homogeneity of the film.

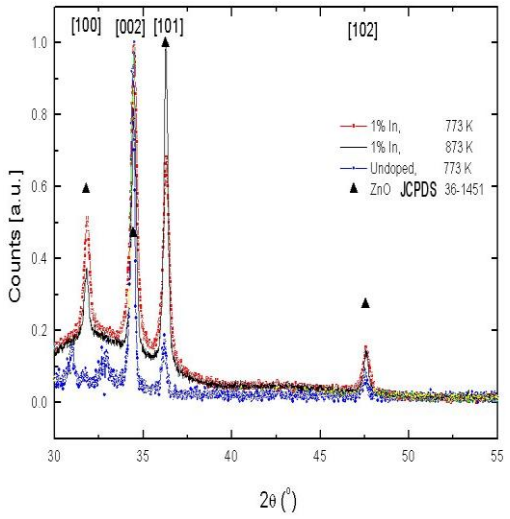

Fig.1. XRD of undoped and 1%In doped ZnO at different substrate temperatures T_s and the same deposition time t_d = 30 min.

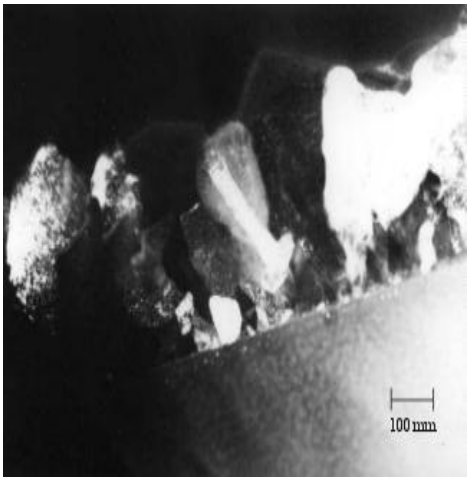

Fig.2. TEM of ZnO:1%In at substrate temperature T_s = 873 K and deposition time t_d = 30min.

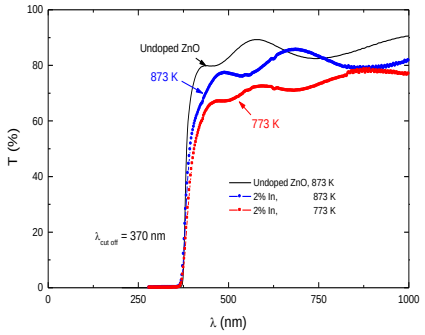

Fig.3. T-λ Curve of undoped ZnO and 2%In doped at different substrate temperatures T_s and the same deposition time t_d = 30 min.

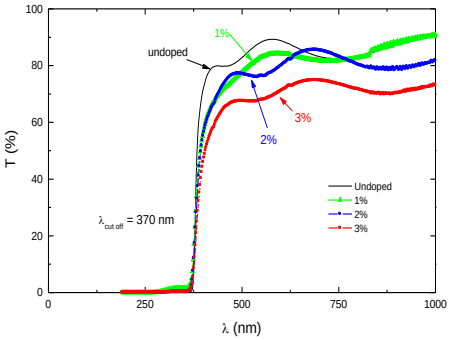

Fig.4. T-λ Curve of undoped ZnO and Indium doped at substrate temperature T_s = 873 K, deposition time t_d = 30min. and different dopant concentrations.

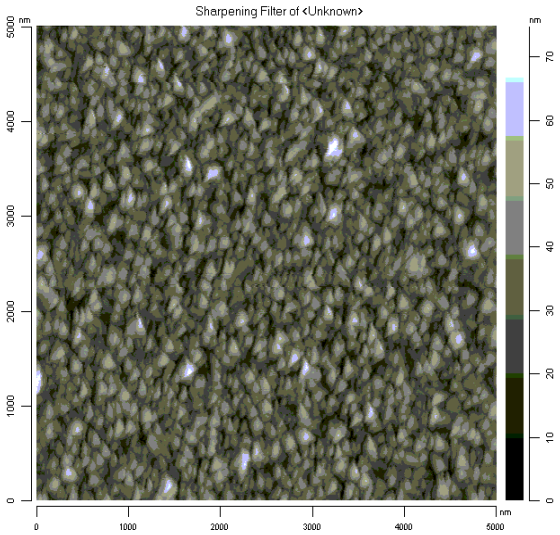

Fig.5. AFM of ZnO:1%In at substrate temperature T_s = 873 K and Deposition time t_d = 30min.

4. Discussion

General examination of XRD patterns of the ZnO:In films, shows that the principal lines [100], [002] and [101] of the hexagonal Wurzite ZnO are the strongest in the whole spectrum (Fig. 1), and correspond very well with the standard patterns of ZnO powder (JCPDS data file 36-1451). No additional peaks could be observed due to the doping with Indium up to 3% in the starting spraying solution. This is due to the low concentration of the dopant (In).

The segregation of Indium at the grain boundary (GB) and the formation of other phases mainly depend on its concentration. In this work, the actual Indium concentration in for example ZnO:1%In was ≈ 0.6% as given by EDX measurements, therefore, the probability for Indium to form other phases or to segregate at grain boundaries is much smaller than to be as interstitial or substituted in ZnO lattice. The appearance of only the characteristic peaks of pure ZnO in XRD patterns supports the conclusion that no other phases are present.

The average size of the micro crystallites can be calculated from the full width at half maximum (FWHM), using the Scherrer formula for crystallite size broadening of diffraction peaks [12]:

$$D = 0.94\,\lambda / \Delta(2\theta)\cos(\theta) \qquad (1)$$

Where $\Delta(2\theta)$ is the full peak width at half maximum of the diffraction line in radians, θ is the diffraction angle and λ is the wavelength of X-rays in Angstrom (Cu K_α, λ = 1.5406 Å).

The texture coefficient TC (hkl) is used as a tool to determine the preferred orientation, which is calculated from the XRD results as follows [13]:

$$TC(hkl) = \frac{I(hkl)/Io(hkl)}{I/N\sum_N I(hkl)/Io(hkl)} \qquad (2)$$

Where I is the measured XRD peak intensity, I_o is the corresponding intensity given in JCPDS data file for the powder, and N is the number of reflections.

Fig. 1 shows the variation of the X-ray diffraction peaks as a result of increasing the substrate temperature of ZnO:1%In films from 773 to 873 K, the [101] peak intensity increases compared to the [002] peak, as the preferred orientation for undoped ZnO. This finding is reported also in literature [1,2], this may be attributed to indium atoms which may be impeding the growth of ZnO in the [002] direction at the applied deposition conditions.

Using Transmission Electron Microscopy TEM (see Fig. 2.), it could be shown that ZnO:In films consist typically of relatively large crystals, some of them have a crystallite size more than 160 nm, and with film thickness of about 270 nm. The same thickness value has been calculated for the same sample from the optical T-λ data. The crystals are well oriented along c direction, which is consistent with data obtained from XRD too. This large crystallite size combined with good orientation indicates that spray pyrolysis technique may be used to produce reasonably large single crystals [14-18]. The calculated crystallite size by Scherrer formula, is much smaller than those measured by TEM. This difference may be attributed to inconsistency of applying the Scherrer-method, which gives the average crystallite size over the plane, such as [002] .

The EDX-investigations of ZnO:1%In film showed that: Oxygen ≈ 49%, Zinc ≈ 49% and Indium ≈ 0.6% (the rest ≈ 1.4% attributed to the by-products and the substrate components).

As the substrate temperature T_s for ZnO:In increased, the transmittance is improved (Fig.3), and this can be partially attributed to the decreasing of the thickness from 420 nm to 350 nm as the temperature increased from 773 K to 873 K, table 1. At higher substrate temperatures the residual organic species (e.g. acetate) evaporate as volatile gases, the deposited crystallites become closer to each other allowing film densification on the expense of the film thickness. Also, there is a possibility that the upward heat flux may impede the incident sprayed fog that induces a decrease in the deposited layer thickness. The re-evaporation of the deposited material at higher substrate temperature is another probable cause for the thinning of the deposited film. The doping of ZnO with Indium lowered the transmittance from almost 90% for undoped to about 70% for the 3% Indium doped, see Fig.4.

ZnO films undoped and doped with Indium, at the same preparation conditions, have similar cut off wavelength $\lambda_g \approx 370$ nm, which corresponds to an optical energy gap $E_g \approx 3.3$ eV, see Fig.4.

Despite of the change of substrate temperature and doping concentration, all the (T-λ) curves of the investigated samples showed the same shape of the uv-absorption edge. This may be due to the small concentration of Indium in the films, as prooved by EDX.

Table 1. Thickness and Refractive Index (n) of Undoped and Indium Doped ZnO

	Composition ZnO	Depos. Temp. T_s [K]	Depos. Time t_d [min.]	T% visible range	thickness [nm]	n
1	Undoped	873	30	90	290	1.9
2	ZnO:2%In	873	30	85	350	1.9
3	ZnO:2%In	773	30	80	420	2.0
4	ZnO:2%In	873	20	90	230	1.9
5	ZnO:2%In	873	40	85	430	1.9
6	ZnO:2%In	873	60	80	630	1.9
7	ZnO:3%In	873	30	75	310	2.0

By using the mentioned software package (APAS) the thickness and refractive index (n) can be calculated from the T-λ curves, Table 1. The estimated refractive index for undoped and Indium doped ZnO was similar ($n \approx 2$).

The atomic force microscopic picture AFM of ZnO:1%In can be seen in Fig.5, generally ZnO:In films are homogeneous and smooth.

By doping ZnO films with Indium it was possible to reduce the sheet resistance R_s from MΩ to kΩ, see Table 2.

Table 2. The sheet resistance and film thickness of undoped and doped ZnO

Compos. ZnO	T_s K	t_d min.	Thick. nm.	R_s KΩ
Undoped	873	30	290	1000
1%In	873	30	290	105
2%In	873	30	350	7
2%In	773	30	420	28
2%In	873	20	230	36

It has been observed that the sheet resistance of the ZnO:In films decreases with increasing doping percentage. The resistance of the films has a minimum value at around 2.0 wt % In. For higher doping levels the sheet resistance starts to increase again. The decrease in the resistance may be explained as the indium atoms doped into ZnO lattice act as donors by supplying a single free electron when In^{3+} ions occupy Zn^{2+} ion sites. This in turn increases the free carrier concentration and at the end decreases the sheet resistance [19]. But with higher doping (> 3 wt%), a recombination process happens which induces an increase of the resistance.

Conclusion

Spray pyrolysis technique has been proved to be an effective tool to produce well oriented undoped and indium doped zinc oxide thin films onto glass substrates. The general examination of XRD pattern of undoped and indium doped ZnO films prepared by spray pyrolysis shows, that they consist of crystals with hexagonal Wurzite structure. The [100], [002] and [101] reflexes belong to the strongest in the diffractograms. ZnO doping with Indium up to 3% causes no additional peaks. By choosing of higher substrate temperatures during the deposition process, the peak intensity increases in the direction [101] compared to the [002] direction, which is the preferred orientation for undoped ZnO. The TEM studies revealed that the films consist of considerably large crystals, some of them with crystallite size more than 160 nm, and with a film thickness of about 270 nm. Similar thickness value could be derived by applying optical methods (~ 280 nm) for the same sample. This means that both methods are compatible for thickness determination. The observed large crystals indicate that spray pyrolysis technique may be used to produce single crystals. Further investigations are on the way. As the substrate temperature T_s for ZnO:In increased - the transmittance is improved. The atomic force microscopy indicates that the ZnO:In films are homogeneous. They have relatively low sheet resistance R_s in the kΩ range compared with undoped ZnO films, which have resistance values R_s in the range of MΩ.

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Histological, Histochemical and Biochemical Studies of the Effect of Chronic Exposure of Noise Stress on the Kidney of Albino Rats

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Abstract: Twenty-five adult male albino rats were utilized to study the effect of noise stress for short and long durations on the kidney structure and functions of albino rats. After one week (short duration) of noise exposure, the biochemical data revealed a significant increase in serum albumin accompanied by a significant increase of body and kidney weights. A significant decrease of urine output was also detected. The renal tufts of the glomeruli were enlarged with increased cellularity filling the Bowman's space. The lining epithelium of the convoluted tubules was slightly swollen. After two weeks of rest, most of the glomeruli and the tubular elements were more or less like those of the control group. After two weeks (long duration) of noise exposure, the biochemical data revealed the same results as those shown in the animals which were exposed to noise for a short duration. However, the body weight of the animals revealed a highly significant increase accompanied by a highly significant decrease in the urine output. The glomerular capillaries of renal corpuscles were swollen, lobulated with increase of the mesengial matrix. The epithelial cells lining renal tubules exhibited cloudy swelling and vacuolar degeneration. After two weeks of rest, the majority of renal glomeruli revealed extensive glomerular retraction and degeneration. The epithelial cells lining the renal tubules showed necrotic changes and their lumina appeared packed with renal casts and cell debris. The thickened basement membranes surrounding both the Bowman's capsules and renal tubules exhibited an intense PAS reaction in rats exposed to long duration of noise stress. After one week of noise exposure, mercury-bromophenol blue staining of the kidney revealed a high protein content in the form of an intense blue colouration of the intact brush borders of the proximal convoluted tubules.
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Key words: Histological, Histochemical, Biochemical Studies, Noise Stress, Kidney, Albino Rats.

Introduction

Recently, many new forms of stresses and pollutants appeared in the world. Noise is one of the most modern and important stressful stimuli encountered nowadays especially in industrialized societies **(WHO, 2010).**

Noise is typically defined as an unwanted sound or a combination of sounds that may adversely affect people. Noise can induce physiological harm or pathological damage. The mechanism of damage from noise has yet to be fully understood, but research has demonstrated a multitude of factors including increased oxidative stress, vascular changes **(Evans et al., 1998 ; PHSHP report, 2000).**

There have been multiple studies that have investigated the relationship between noise, blood pressure, and myocardial damage **(Babisch, 2003; Sangeeta et al., 2009; Bao, 2011).** Other studies investigated the relationship of noise and blood pressure and stress response of cardiovascular system **(Evans et al., 1998 ; Wolfgang & Irene 2009; Kao et al., 2010)**.

Numerous studies recorded different pathological changes in renal functions following different types of stress **(Ahmed & Mazher, 2006; Moussa, 2005; Siems et al., 2001; Danielski et al., 2003)**. However, a little attention was paid to throw the light on the histological alterations that may occur in the kidney after chronic exposure to noise stress. Thus, the present study aims to determine the effects of exposure to noise stress for two different durations on the kidney histology, histochemistry and some biochemical parameters.

Material and Methods

Forty-two adult male albino rats (Rattus norvegicus), each weighing 130 $^{+}$ 10 grams were utilized in the present study. The animals were housed in specially designed wooden cages and were kept under similar constant airflow and at a convenient temperature during the whole period of experimentation. Rats were fed on a balanced laboratory diet, while water was allowed ad libitum.

The animals were divided into three groups, as follow **:**

Group I: This group included 6 animals used as control.

Group II: This group included 18 animals exposed to noise stress by using a special record to produce a

continuous noise of 100 decibels for 8 h / day for one week.

Group III: This group included 18 animals exposed to the same dose of noise stress (100 decibels for 8 h / day) for two weeks.

The last two groups were subdivided into two subgroups according to the time of sacrifice, where the animals of first subgroup were sacrificed immediately after the end of the period of noise exposure while animals of the second subgroup were sacrificed two weeks following the end of noise exposure.

Biochemical studies:

The body and kidney weights were recorded just before the beginning of study (day 0) and at the termination of the study (29 days).

Before scarifications, rats were individually housed in metabolic cages and urine was collected for estimating its volume (output/day).

Biochemical analysis of serum albumin was estimated according to **Drupt (1974).**

Histological study:

After fast dissection, the kidneys were rapidly excised and quickly trimmed into small pieces that were fixed in Carnoy's fixative and processed until paraffin blocks. Sections of 6 µm were prepared and stained with Harris haematoxylin and eosin **(Drury & Wallington, 1980)** to illustrate the histological alterations of the kidney.

Semi-quantitative analysis:

Histological alterations were scored in 100 fields/each group. Ten of them were randomly selected at 4oo magnification/each examined animal. The results were scored as percentage of the damaged tubules in the field examined as follows:

Mild damage: areas of tubular damage <25%.

Moderate damage: areas of tubular damage equalling 25%.

Severe damage: areas of tubular damage >50 %.

The presence of luminal debris, hyaline casts, cytoplasmic vacuolization and nuclear changes were used as evidence on tubule damage **(Shah & Walker, 1988).** Semiquantitative measurements were carried out using image analyzer (Super Eye-Heidi Soft), Diagnostic Pathology Department, Jubail Royal Hospital, Kingdom of Saudi Arabia.

Histochemical study:

For the histochemical study, small pieces of kidney were fixed in Carnoy's fluid and then processed and sectioned to get 6 µm thick paraffin sections. Then, the following methods were applied:

1. Periodic acid Schiff's (PAS) technique for the demonstration of polysaccharides **(Hotchkiss, 1948).** The nuclei counterstained by Harris haematoxylin.

2. Mercury bromophenol blue technique for the demonstration of total protein **(Mazia et al., 1953).**

Statistical analysis:

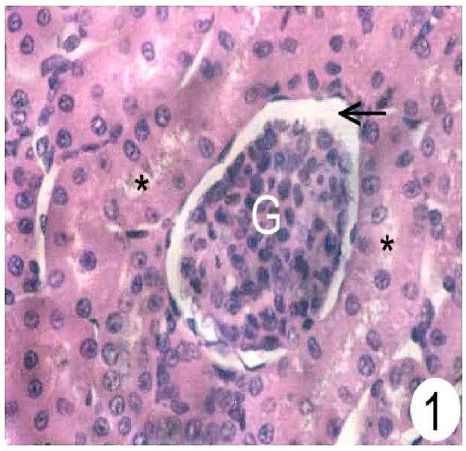
The statistical data included body weight, kidney weight, urine output and serum albumin level**.** Data were expressed as arithmetic mean $^{+}$ standard deviation (SD) .Student t-test was used to test the significant change of each parameter of exposed animal in comparison to control group. Statistical analysis of the data was performed by Med Cale software for medical statistics **(Schoonjans et al., 1995).**

Results

Histological Observations:

Group I (Control):

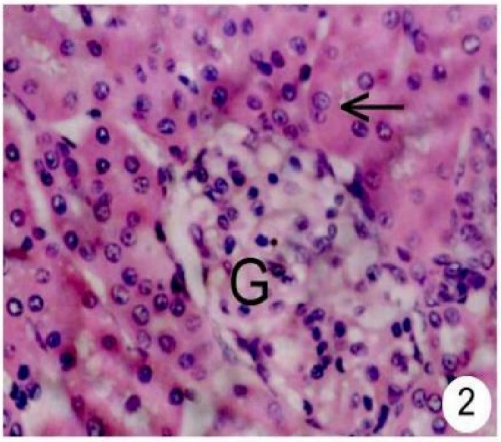
The glomeruli of the control rats showed a normal histological picture, where both visceral and peripheral layers of renal capsules were clearly identified and the Bowman's spaces were clearly visible. The lining epithelia of both the convoluted and collecting tubules were normal in shape, having granular acidophilic cytoplasm and normal nuclei **(Fig. 1).**



(Fig.1): A photomicrograph of a kidney section a control rat showing the normal histological configuration of the kidney. The photo shows a glomerulus **(G)**, Bowman's space **(arrow)** and the lumina of the proximal convoluted tubules **(*)**. **(H&E Stain X400)**

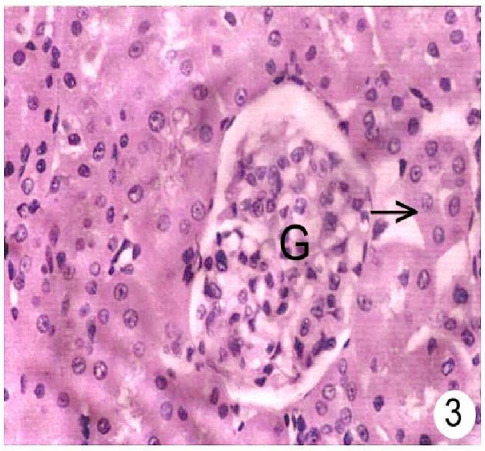
Group II*:*

In animals of subgroup A which were sacrificed immediately after the end on noise exposure, showed that the glomerular tufts were enlarged with increased cellularity to fill the Bowman's space. The lining epithelium of the convoluted tubules was slightly swollen and showed smudgy appearance. Some of the cells of such tubules lost their boundaries and brush borders **(Fig. 2).**



(Fig.2): A photomicrograph of a kidney section of a rat after one week of exposure to noise stress **(of subgroup II A)** showing an enlarged glomerular tuft **(G)** filling Bowman's space and the swollen epithelial cells **(arrow)** lining the proximal convoluted tubules. **(H&E Stain X400)**

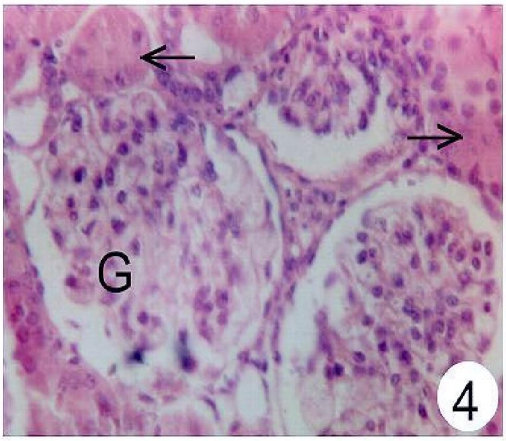
In animals of subgroup B, most of the glomeruli and the elements of the kidney tubules were more or less similar to those of the control group. However, few areas of renal cortex revealed some dilatation of Bowman's spaces **(Fig. 3).**



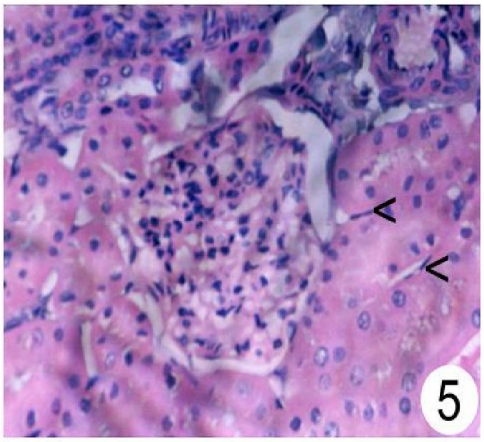
(Fig.3): A photomicrograph of a kidney section of a rat exposed to short duration of noise stress **(of subgroup II B)** showing a more or less normal glomerulus **(G)** and epithelial cells lining the convoluted tubules **(arrow). (H&E Stain X400)**

Group III*:*

In animals of subgroup A, the histological examination revealed high cellularity, lobulated and congested glomeruli. The glomerular capillaries of renal corpuscles were swollen and displayed increase of the mesengial matrix that showed adhesion to the visceral cells of Bowman's capsules. The epithelial cells lining the convoluted and collecting tubules of both cortex and medulla exhibited cloudy swelling and vacuolar changes **(Fig. 4).** Moreover the epithelial cells lining the proximal convoluted tubules lost their boundaries and had damaged brush borders. Numerous compressed endothelial cells were observed in the interstitial spaces inbetween the renal tubules **(Fig. 5).**



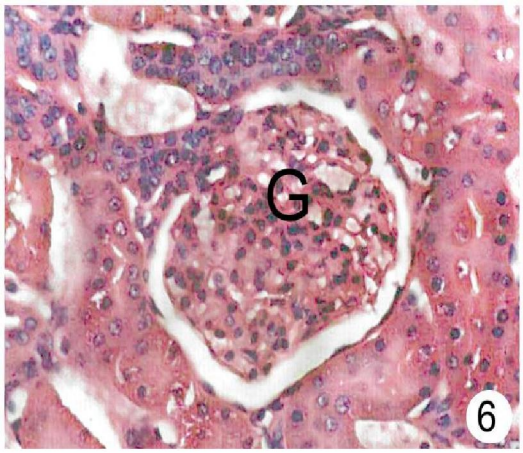
(Fig.4): A photomicrograph of a kidney section of a rat exposed to long duration of noise stress **(of subgroup III A)** showing swollen, lobulated and congested glomeruli **(G)** and swollen epithelial cells lining the convoluted tubules **(arrow). (H&E Stain X400)**



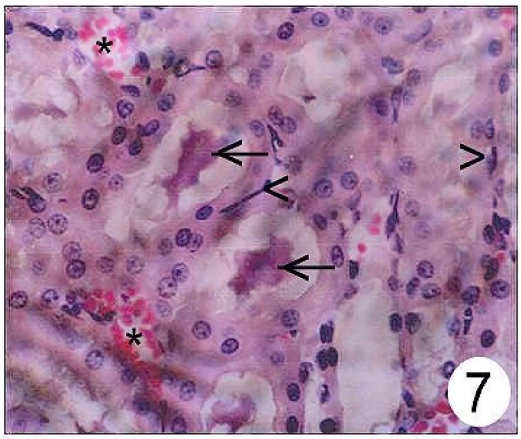
(Fig.5): A photomicrograph of a kidney section of a rat exposed to long duration of noise stress **(of subgroup III A)** showing that the epithelial cells lining the proximal convoluted tubules lost their boundaries and numerous compressed endothelial cells **(arrowheads)** are shown inbetween the renal tubules. **(H&E Stain X400)**

In animals of subgroup B, the majority of renal glomeruli revealed extensive glomerular retraction or atrophy with extremely widened Bowman's spaces**.** Also, some glomeruli showed local degeneration**.** The epithelial cells lining the

convoluted and collecting tubules were partially detached and showed necrotic changes **(Fig. 6)**; their lumina appeared packed with renal casts and cell debris. Numerous compressed endothelial cells, blood cells and haemolysis were observed between the renal tubules **(Fig. 7)**.



(Fig.6): A photomicrograph of a kidney section of a rat exposed to long duration of noise stress **(of subgroup III B)** showing a retracted glomerulus **(G)** and a wide Bowman's space **(arrow). (H&E Stain X400)**



(Fig.7): A photomicrograph of a kidney section of a rat exposed to long duration noise stress **(of subgroup III B)** showing the collecting tubule's lumina packed with renal casts **(arrows)** , numerous compressed endothelial cells **(arrowheads)** and blood cells between the renal tubules(*).**(H&E Stain X400)**

Semiquantitative results:

Table (1): Semi-quantitative analysis of renal histological alterations.

Group / Parameter	control	Group II a	Group II b	Group III a	Group III b
No Change	95	77	92	21	18
Mild Change in the tubule	5	20	6	61	22
Moderate Change in the tubule	0	3	2	14	31
Severe in the tubule	0	0	0	4	29
Total	100	100	100	100	100

Histochemical results:

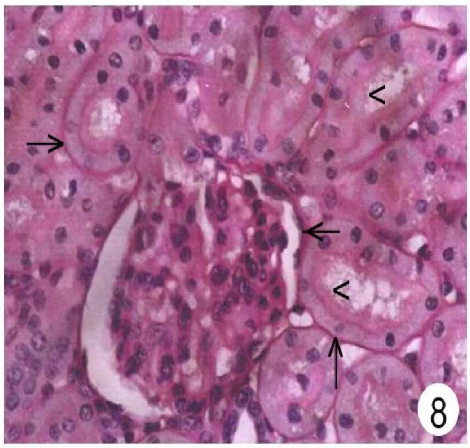
PAS reaction:

Group I (Control):

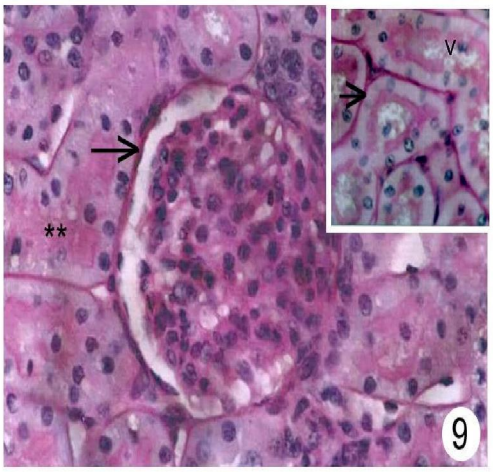
The PAS-stained sections revealed purple colour of the brush border of the proximal convoluted tubules and basement membranes of both Malpighian corpuscles and all the renal tubules **(Fig. 8)**.

Group II:

In animals of subgroup A, many of the proximal convoluted tubules appeared with intact brush borders; however some of them showed that PAS-positive brush borders were partially lost. A slight increase was observed in the thickness of the basement membranes surrounding both the Bowman's capsules and the renal tubules **(Fig.9)**.

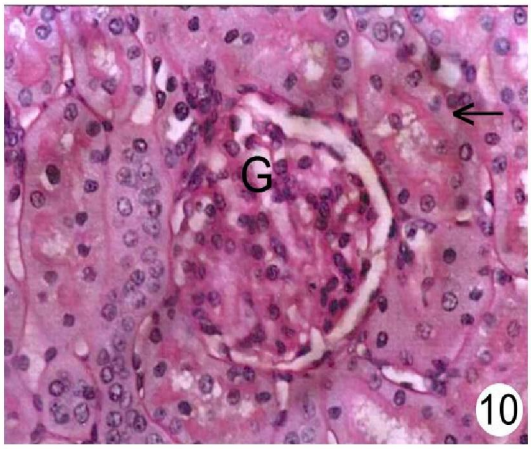


(Fig.8): A photomicrograph of a kidney section of control rat showing the normal PAS-positive brush border of the proximal convoluted tubules **(arrowheads)** and basement membranes **(arrows)**. **(PAS reaction counter with H&E stain X400)**



(Fig.9): A photomicrograph of a kidney section of a rat after one week of exposure to noise stress **(of subgroup II A)** showing intact brush borders of some proximal convoluted tubules **(**);** others lost brush borders **(arrowheads)** and display slightly thickened basement membranes **(arrows)**. **(PAS reaction counterstained with H&E stain X400)**

In animals of subgroup B, the PAS-positive materials of both the renal glomeruli and the renal tubules elements appeared more or less similar to those of the control group **(Fig. 10)**.



(Fig.10): A photomicrograph of a kidney section of a rat exposed to short duration of noise stress **(of subgroup II B)** showing a more or less normal distribution of the PAS-positive materials of both the renal glomeruli **(G)** and the renal tubules **(arrow). (PAS reaction counterstained with H&E stain X400)**

Group III:

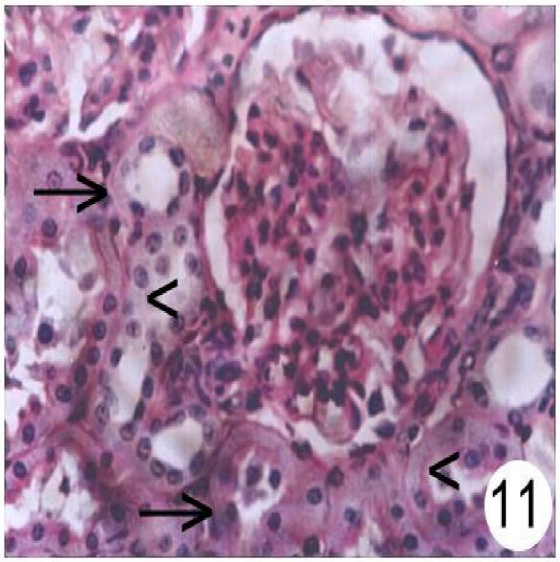
In animals of subgroup A, the PAS positive reaction showed that the brush borders of most of the proximal convoluted tubules were completely lost and their basement membranes appeared faintly stained and ill-defined as compared with the control sections **(Fig. 11)**.

In animals of subgroup B, the PAS-stained sections showed a strong PAS reaction of the thickened basement membranes surrounding both the Bowman's capsules and the renal tubules as compared with the control sections **(Fig.12)**. Moreover, considerable deposits of hyaline materials were detected in the collecting tubules and/or interstitial tissues **(Fig.13)**.

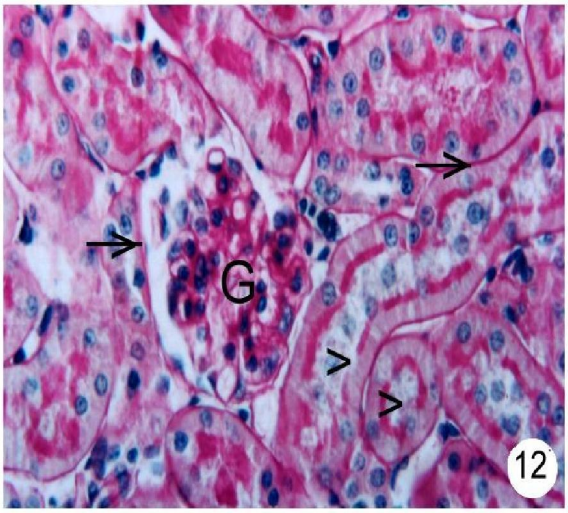
Mercury bromophenol blue staining:

Group I (Control):

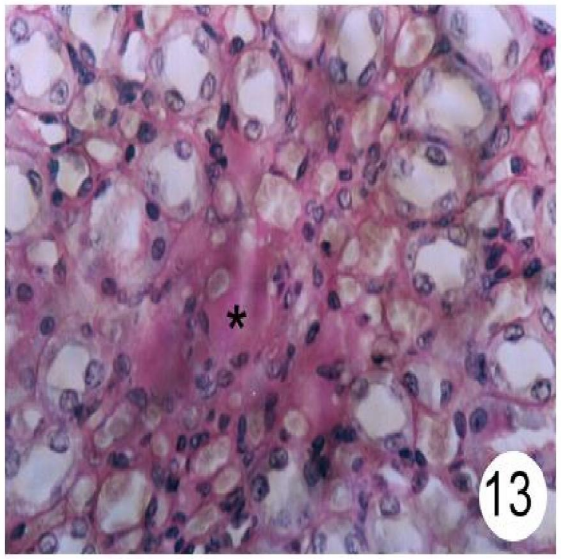
The renal glomeruli displayed moderate protein content. The epithelium lining the renal tubules display a dark blue colouration of proteinic particles diffused homogeneously in the cytoplasm of their cells **(Fig. 14).**



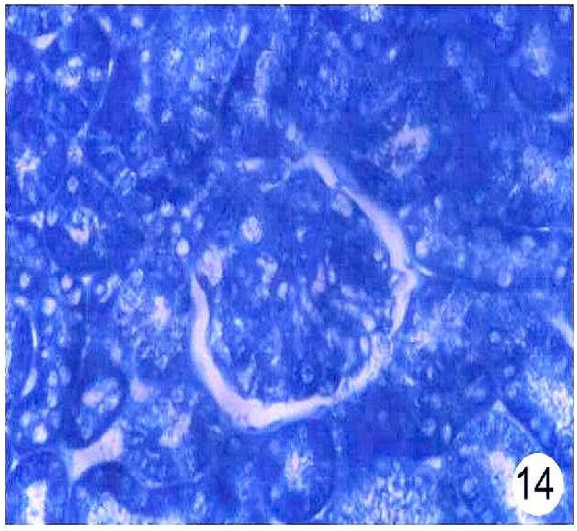
(Fig.11): A photomicrograph of a kidney section of a rat after two weeks of exposure to noise stress **(of subgroup III A)** showing the degenerated brush borders of most of the proximal convoluted tubules **(arrowheads)** and ill-defined basement membranes **(arrow). ((PAS reaction counterstained with H&E stain X400)**



(**Fig.12):** A photomicrograph of a kidney section of a rat exposed to long duration of noise stress **(of subgroup III B)** showing a strong PAS-reaction of the thickened basement membranes surrounding both the Bowman's capsules **(arrow)** and the proximal convoluted tubules **(arrowheads)** which lost the brush borders**. (PAS reaction counterstained with H&E stain X400)**



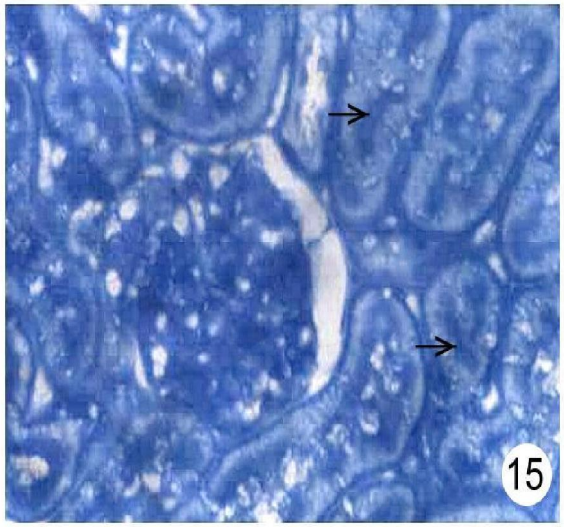
(Fig.13): A photomicrograph of a kidney section of a rat exposed to long duration of noise stress (**of subgroup III B)** showing deposits of hyaline materials **(*)** in collecting tubules and/or interstitial tissues. **(PAS reaction counterstained with H&E stain X400)**



(Fig.14): A photomicrograph of a kidney section of a control rat showing the normal distribution of total protein contents**. (Mercury bromophenol blue method X400)**

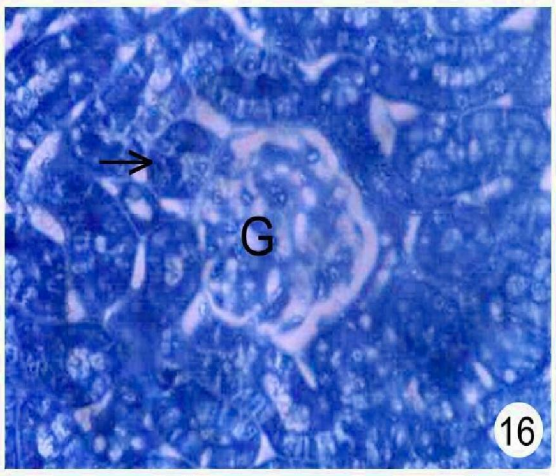
Group II:

In animals of subgroup A, the glomerular tufts and the epithelial cells lining the renal tubules showed a slight decrease in their proteinic contents. Many of the proximal convoluted tubules appeared with intact brush borders revealing an intense dark blue colouration **(Fig. 15).**



(Fig.15): A photomicrograph of a kidney section of rat after one week of exposure to noise stress **(of subgroup II A)** showing a slight decrease in their proteinic contents and the intact brush borders **(arrow)** of the proximal convoluted tubules, these display an intense blue colour. **(Mercury bromophenol blue method X400)**

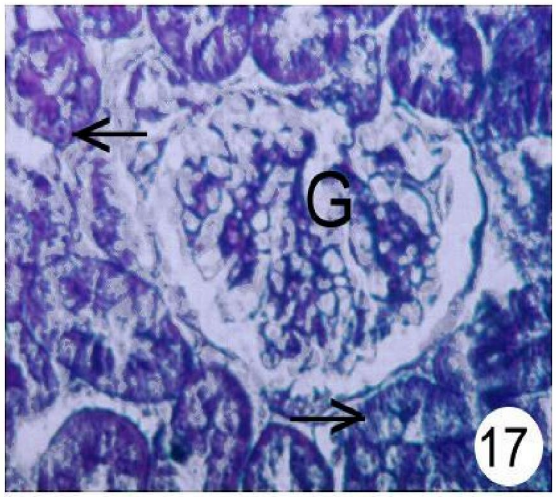
In animals of subgroup B, the stained proteinic materials of both the renal glomeruli and the renal tubules elements appeared more or less like those of the control group **(Fig. 16).**



(Fig.16): A photomicrograph of a kidney section of a rat exposed to short duration of noise stress **(of subgroup II B)** showing almost normal distribution of the positive total protein materials of both the renal glomeruli **(G)** and the renal tubules **(arrow). (Mercury bromophenol blue method X400)**

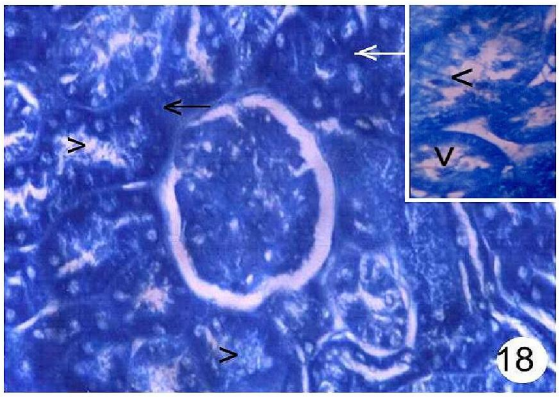
Group III:

In animals of subgroup A, a marked decrease in the proteinic contents was observed in both the glomerular tufts and the epithelial cells lining the renal tubules. These cells appeared faintly stained, vacuolated and pocessed dark blue intensely-stained pyknotic nuclei **(Fig.17)**.



(Fig.17): A photomicrograph of a kidney section of a rat after two weeks of exposure to noise stress **(of subgroup III A)** showing a marked decrease in the proteinic contents in both the glomerular**(G)** tufts and the vacuolated epithelial cells lining the renal tubules **(arrow). (Mercury bromophenol blue method X400)**

In animals of subgroup B, the sections revealed a strong stainability with bromophenol blue in the atrophied epithelial cells line both the collecting and convoluted tubules. Darkly blue stained granular casts and cell debris were accumulated in the lumina of the proximal convoluted tubules and in the interstitial tissue **(Fig.18)**.



(Fig.18): A photomicrograph of a kidney section of a rat exposed to long duration of noise stress **(of subgroup III B)** showing a strong stainability of total protein contents in the atrophied renal epithelial cells **(arrows) and** granular casts and cell debris in the lumens of renal tubules **(arrowheads)** . **(Mercury bromophenol blue method X400)**

Table (2): The biochemical statistical data of rats exposed to noise stress.

Group Parameter	control	*Group II* a	*Group II* b	*Group III* a	*Group III* b
body weight/g	110 ± 10	127 ± 6*	119 ± 8	139 ± 67**	143 ± 2**
Kidney weight/g	1.28 ± 0.4	1.38 ± 0.3*	1.23 ± 0.9	1.51 ± 0.4**	1.1 ± 0.2*
urine out put/ml/100g b. w./day)	5.4 ± 0.5	4.2 ± 0.4*	5.1 ± 0.2	4.6 ± 0.3*	4.4 ± 0.3*
Albumin g/dl	3.1± 0.5	2.2 ± 0.4*	3.2± 0.1	2.0± 0.4*	1.51± 0.2**

All data are Mean value± Standard error. N= 6 animals of each group.
* Significant at ($p < 0.05$). ** Highly significant at (< 0.01).

Discussion

Although numerous investigations have been carried out on different pollutants, noise stress is the least illustrated or understood. The different pathological changes produced by noise are governed by sympathetic nervous system and appear to be similar to those produced by other physical and chemical stresses **(Dejoy 1984; Kjellberg 1990).**

Initially under the conditions of the present study, both glomeruli and renal tubules seemed to be sensitive to the noise stress. However, the renal tubules largely returned to the normal histological pattern in rats which were exposed to noise for one week (short duration) after two weeks of rest following the end of exposure. This recovery probably occurred because of active tubular regenerative processes, whereas the glomeruli lesions persisted and evolved towards some membranous changes in case of long exposure periods **(Marino & Becker 1977).**

The reversibility of renal changes was not detected when exposure to noise stress has been omitted for two weeks. This is based on, multiple doses of noise exposure at a constant value of intensity is not completely reversible and appeared more or less to be permanent due to severe degenerative changes which are difficult to be completely recovered.

Another explanation is that prolonged exposure to stressful stimuli may have caused prolonged rise in blood pressure leading to circulatory adaptation and permanent hypertension **(Andren et al., 1980; Pereet, 1982; Van Kempen, et al., 2002).**

In view of the present results, it was found that the experimental animals which were exposed to noise stress for two weeks (long duration) did not reveal signs of reversibility after two weeks of rest following the end of exposure. The kidney's tissues still showed advanced degree of glomerular shrinkage or collapse, renal tubules damage, in addition to the presence of interstitial blood cells and some areas of haemolysis.

Nekhoroshev & Glinchikov (1991) recorded micropunctate hemorrhages under the effect of white noise stress for 7 days.

Also, **Van Dijkken, et al. (1992)** reported that animals exposed to chronic stress showed congestion and haemorrhage in all the body organs due to permanent rise in arterial blood pressure. Thus, the present histological alterations-that attributed to a sustained elevation of blood pressure-may be associated with the risk of hypertension.

Some other studies have suggested that occupational noise exposure is associated with a sustained elevation of blood pressure **(Nawaz &Hasnain 2010 ; Tomei et al., 2010)** or with a higher risk of hypertension **(Sbihi et al., 2008),** but other studies have not revealed any significant interaction **(Talbott et al., 1985 ; Inoue et al., 2005).**

Andren et al. (1979) proved that noise influences the renal handling of salts. This effect was believed to be due to an isolated secretion of oxytocin without concomitant secretion of vasopressin. Moreover, **Koepoke, et al., (1988)** and **Koepoke (1989)** stated that environmental stress increases renal sympathetic nerve activity and decreases urinary sodium excretion in both normotensive and hypertensive rats through increased renal tubular reabsorption of sodium and water.

Other studies postulated the cause of renal changes after noise exposure; yet, the most acceptable one was the occurrence of hypertension. This hypertension is believed to be due to the elevation of suprarenal hormones level.

Noise as a stressor leads to increased release of the stress hormones including epinephrine, nor-epinephrine and adrenal steroids**,** thus, causing elevation of sodium- water retention and increase the arterial blood pressure **(Andren et al., 1979; Brandenberger et al., 1980; Lai &Carino, 1990).** Moreover, **Babisch (2003) and Seema et al. (2010)**

recorded that acute and chronic exposure to noise stress causes changes in neuroendocrine, psychological and behavioral functions.

Swollen renal tubules with intact or damaged brush borders revealed a slight decrease in PAS reaction. However, the thickened basement membranes surrounding both the Bowman's capsule and renal tubules showed intense PAS reaction.

It is well known that renal blood flow is decreased during stress and it is equally recognized that this ischemia is due to renal arterial vasoconstriction **(Dastron &Deutsches, 1975).** Thus, the advanced degree of glomerular shrinkage or collapse and the damages of the renal tubules may also be attributed to ischemia.

Hypoxia, causing decrease in the cellular ATP and associated with an increase in cAMP, could stimulate the phosphorylase enzyme activity responsible for glycogenolysis in order to maintain the cell's energy sources by generating ATP from glycogen **(Cotran et al., 1994).**

Moreover, the hormonal changes reported on exposure to noise stress could cause increased secretion of adrenaline and noradrenaline which in turn increases glycogenolysis and / or inhibits glycogenesis **(Dixey&Rein, 1982)**

The biochemical measurements of the present study showed that both the relative body and kidney weights of noise exposed animals for one week displayed a significant increase ($p < 0.05$) than that of the control group. These increased values gradually returned to the normal level after two weeks of rest. However the relative body and kidney weights of noise exposed animals for two weeks showed a highly significant increase ($p < 0.01$) than that of the control group. Such increases of body and kidney weights seemed to be proportional with the decreased levels of urine output of the same animal groups. These changes may also be attributed to the elevation of blood pressure which increases the water and sodium retention.

On the contrary, the kidney weight did not return to the normal value after two weeks of rest in animals which were exposed to noise to a long duration but showed a significant decrease ($p < 0.05$) as compared to the control rats. Also, the urine output still revealed a significant decrease ($p < 0.05$) value as compared with that of the control rats. These changes may be attributed to the prolonged elevation of blood pressure which in turn affects the kidney tissues leading to renal atrophy. Similar results were recorded by **Bennett et al. (1996)** in cases of renal toxicity. They related these changes to tubular atrophy and tubule-interstitial ischemia.

The advanced degrees of glomerular and tubular damage depicted in the present study support this explanation, where the glomerular injury was proportionate to the severity of the hypertension **(Fujihara et al., 1994).**

The increase of thickness of basement membranes depicted in the present study may be attributed to glomeruli and renal tubules shrinkage or collapse, as the same amount of basal lamina came to accumulate in a smaller volume of retracted tissues **(Ghadially, 1982).**

The biochemical measurements of serum albumen showed significant decreases ($p < 0.05$) in both animals exposed to noise stress for short and long durations. This decreased levels accompanied with a significant decrease in the body weights of animals of the same groups. This significant decrease ($p < 0.05$) was also accompanied with a similar decrease in the urine output.

The serum albumin level returned to the relative normal value after two weeks of rest in animals exposed to noise for short duration. However, excessive loosing of albumin was recorded in animals exposed to noise for long duration and showed highly significant decrease values ($p < 0.01$) as compared with the control group.

In this respect, **Howie (1986)** suggested that the glomerular adhesions with Bowman's capsules are secondary to the lack of proteins or to some factors associated with their synthesis. Moreover, **Lawrence& Brewer (1982)** found that increased albumin glomerular filtration was shown to cause fusion of glomerular epithelium in female rats with hyperalbuminurea. They postulated that protein droplets acts by stimulating glomerular epithelial cells endocytosis with the result that these protein droplets accumulate within the epithelial cells so causing marked cytoplasmic swelling which forces the glomerular foot processes to spread out and fuse together.

Glassock (1985) reported that the glomeruli from heavily proteinuric rats showed mesengial cells hypercellularity and mesengial matrix expansion. Thus, the glomerular adhesion may be attributed to certain damage of the glomerular cells which may lead to increased influx of macromolecular substances into glomerular mesengium causing overload of mesengial matrix. So, **Neill et al. (1991)** and **Bains et al.(1997)** stated that glomerular injury can be identified functionally as proteinuria, particularly in the presence of high molecular weight proteins which are normally non-filterable. Thus, whenever there is heavy proteinuria, the glomerular epithelial cells show dramatic morphological changes which clearly demonstrate changes in cell adhesion.

The protein staining of the rat's kidney after one week of exposure to noise stress revealed an intense dark blue colouration of the intact brush

borders; this supports the previous opinions. The biochemical test of serum albumin also supports this opinion and indicates the increase of protein losing. Thus, in view of the present study, the histological lesions and histochemical alterations induced by noise stress were in good agreement to the biochemical measurements.

In conclusion, the semiquantitative analysis of renal histological alterations observed in the present study revealed the reversibility of renal changes when the exposure has been emitted for short duration. However, as exposure to noise was emitted to long duration, renal changes appear to be severe and permanent. It is directly and irreversibly affects the renal functions and causes permanent histopathological changes. However, the short duration of exposure insignificantly affects the renal functions and causes temporary and reversible histological alterations. It is recommended that all workers exposed to noise must avoid the continuous exposure to acute noise stress for long durations. Also, they must be periodically subjected to check up for their renal function tests, blood pressure and educated about the value of using ear protectors.

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Field Efficiency and its Use for Energy Coefficient Determination

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Abstract: Field efficiency is the most used factor in determining the effective field capacity but yet not known for Iran. This figure for different machines and field conditions are tabulated in ASBAE standards and cited in many publications as a range for different speeds but; they may not hold true for different conditions in different areas. The field efficiency is also needed for converting fuel used in field operation from l/h into l/ha and many more of the kind. Field experiments were run to determine the field efficiency (FE) by measuring wasted time as well as running time for a moldboard plough, chisel packer, tandem disk, mechanical row planter and pneumatic row planter. The energy coefficient for these implements were also obtained and compared. A mean FE of 73.16%, 75.33, 73.5, 68.16, 73.8 and 64.4 and energy coefficient of 26.36, 14.06, 12.19, 6.64, 4.96 and 7.10 l/ha were obtained for moldboard plough, chisel packer, chisel, tandem disk, mechanical planter and pneumatic planter respectively.
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Keywords: Field efficiency, moldboard plough, chisel packer, tandem disk, energy coefficient

1. Introduction

Field efficiency is the most used factor in determining the effective field capacity but yet not known for Iran. This figure for different machines and field conditions are tabulated in ASAE standards and cited in many publications as a range for different speeds but; they may not hold true for different conditions in different areas. Measurements or estimates of machine field capacities are used to schedule field operations, power units, and labour, and to estimate machine operating costs. Hunt (1995) states that 10 items are involved in time efficiency out of which only 6 are included in field efficiency. Time efficiency is a percentage reporting the ratio of the time a machine is effectively operating to the total time the machine is committed to the operation. Field efficiency within the range of 88-74, 90-77, 90-75 and 78-55% are given for mouldboard plough, disc harrow, chisel plough and row crop planter respectively. ASAE standard D497.4 FEB03 lists a range of field efficiency for many machines. For mouldboard plough, tandem disk harrow, roller packer, chisel plough and row crop planter they are all within the range of 70-90% with typical values of 85, 80, 85, 85 and 65% respectively. Al-Hashem (2000) measured field efficiency to be 20.6%, 18.0% and 23.6% for disc plough, tooth harrow and levelling implement, respectively. These efficiencies are much lower than that recommended by ASAE. Moreover, efficiencies greatly varied among the farms for these implements for each specific operation. Hanna (2002) has given values of 85, 85, 83 and 70 percent for plough, chisel plough, tandem disk and air seeders respectively. He also mentioned that the FE can be taken from the table in his publication or estimated using equation (1) if you have a representative value of EFC (Effective Field Capacity). FE(%)=EFC/TFCx100 (1) where FE is the abbreviation for field efficiency, EFC for effective field capacity and TFC for theoretical field capacity. Helsel et al (2011) stated that machine maintenance and repair affect field efficiency – equipment that is well-maintained and in good condition operates most efficiently. To reduce turning time, farmers should strive to make fields large, long, and narrow by eliminating fence rows, ditches, or other barriers. Larger implements, if matched to tractor size, can be more field efficient because bigger implements cover larger areas and require a smaller number of turns. Al Hamed (2005) measured field efficiency, effective field capacity, fuel consumption per unit area, and specific fuel energy were estimated during tillage operation in sandy loam soil for three chisel ploughs with different shank shapes. For all the three ploughs, the results showed an inverse relationship between field efficiency and forward speed, and field efficiency values were close at each speed of the four forward speeds. Abubakar et al (2009) assessed the effects of soil physico-mechanical properties on performance efficiencies of ox-drawn mouldboard

plough was conducted with the results that FE was highest (0.133 ha/hr) in Sabon-gari and lowest (0.112 ha/hr) in FDF (Futy Demonstration Farm) at the same soil M.C. (25%). Lowest time losses of 13 and 14 minutes was at 10 and 25% soil M.C.in Sabon-gari with relatively lower (1.35 Mgm^{-3}) soil compactions. Grisso et al (2001) compared the fields that are relatively flat with straight rows with contoured fields with slopes up to 3 to 5%. Field efficiency, travel speeds, and unproductive time lapses were compared. When contour patterns were compared with the straight rows, field efficiency dropped on the contours by 10 and 20% for planting and harvesting, respectively. Bochtis et al (2010) monitored and analyzed two machinery operations in two different fields. The results show that the implementation of the CTF (Controlled Traffic Farming) system rather than the UCTF (Uncontrolled Traffic Farming) system significantly increases the in-field transport distance traveled by the application unit. The reduction in-field efficiency, in terms of transport distance, ranged from 4.68% to 7.41%. Peg Zenk (2003) proved that GPS systems can provide all the data you need to monitor equipment efficiency. "What improves field efficiency numbers is long, straight rows, and we've always known that," Taylor says. "But now we can quantify it." He found that increasing planter size will make an operation more productive but less efficient. A grower can do more work with two 8-row planters than one 16-row model, he notes. Ehsani (2010) stated that in the past, calculating factors such as field efficiency was very difficult, time consuming, and required someone with a stopwatch on-site during operation. Now, GPS can be used to obtain this information much faster and simpler. Khan et al (2010) computed direct energy inputs for wheat, rice and barley crops contain human, pumping, tractor or other self propelled machinery and indirect energy sources are seed, fertilizer and plant protection agro-chemical. Maximum energy consumption was on the farms of rice (6699 kWh ha^{-1}) compared to other two crops (wheat 3028 and barley 2175 kWh ha^{-1}). Behroozi Lar et al (2009) obtained the energy coefficient for growing irrigated wheat in four provinces of Iran and concluded that these provinces used 24.10 to 38.98 $GJha^{-1}$ to produce one hectare of irrigated wheat compared to 23.67 $GJha^{-1}$ predicted for the worst case. Singh (2002) obtained 15.29 $GJha^{-1}$ for producing irrigated wheat In dry and hot places in India. El Hussein and Van Ouwekerk (2005) obtained a value of 13.96 $GJha^{-1}$ for wheat production in Morocco. Slotz (2000) extracted data for conventional wheat production for three varieties of wheat with means equal to 18.3, 17.2, 16.5 and 17.33 $GJha^{-1}$ and 3.10 $GJha^{-1}$. Sidhu (2004) obtained 19.58 $GJha^{-1}$ for producing wheat in one of the provinces of India.

2. Material and Methods

Eighteen plots of 67×10 meter were randomly adopted to implement three tillage operations in six replication with randomized completely block design. The tillage treatments were moldboard plough, chisel packer and chisel. Disking operation was performed on each plot after plowing. Two methods of planting that is mechanical row crop planter and Pneumatic row crop planter were used for seeding. Time spent and fuel consumption for tillage operations and planting as well time and fuel consumption for turning at the head lands were recorded. Field efficiency was calculated using the equation (2).

$$FE = \frac{PT}{PT + TT} \quad (2)$$

Where PT is productive time, TT = turning time. Other involved wasted times such as repair and maintenance, loading and etc. were not considered because no such operations were performed. Field capacity was then calculated from equation (3).

$$C = \frac{vwe}{10} \quad (3)$$

Where v is speed in km/h, w = implement working width in meter and e the field efficiency in decimals.

The energy coefficient in l/ha fuel used was determined by dividing the measured fuel use in l/h by the field capacity. The fuel used for machine operation was calculated from equation (4) and machine drawbar power from equation (5) below,

$$l/h = 4.93 + 0.1997 \times 0.75 \times P_{dbm} \quad (4)$$

$$P_{dbm} = \frac{Fv}{3.6} \quad (5)$$

Where P_{dbm} was requirement power for machine operation in kW, F = draft force of the implement in kN. The equivalent draw bar power need for tractor movement calculated form equation (6).

$$p_{dbt} = \frac{fmgv}{3600(1 - S\%)} \quad (6)$$

f was the rolling coefficient against the tractor wheels, m the tractor mass in kg, g the gravitational force= 9.81, v the speed and S% the wheel slip in decimals. The P_{dbt} was added to P_{dbm} in equation (5) prior to calculating the l/h.

3. Results and Discussion

Mean data collected and calculations is shown in table 1. The field efficiency (FE) for ploughs and the mechanical planter with were not significant at 1% probability level. Therefore a mean value of 74% may be used for all and every one of these implements. FE for disking with a value of

0.68 and for pneumatic planter of value 0.63 were significantly different at 1% level which also were differently significant from FE for ploughs. Table 2 shows a comparison of the results with that from different cited references: ASAE, Hunt (1995) and Hanna (2002). The experimental FE for moldboard and chisel plough was about equal to the minimum value given by Hunt but much lower than the typical value of the ASAE and Hanna (2002). For the tandem disk harrow and roller packer, the experimental value was about 15% lower than the one given by ASAE. The experimental row crop planter FE was close to the highest of the Hunt (1995) and about 15% higher than the one given by ASAE. The bar graph for this comparison is shown in figure 1. A bar graph of energy coefficient versus speed for different machines is shown in figure 2. Energy coefficients (EC) for machine operations are also shown in table1. For comparison, the EC cited from Anonymous (2007), Anonymous (2007), Cromwell (1995), Anonymous (2001), Griffth et al (2005) and Molenhuis (2001) is shown in table 3. It seems that the experimental EC for moldboard was at least 1.5 times the maximum given in table 3. This could be due to including the tractor fuel use in experimental calculations while this fuel might have not been accounted for in the figures of table 3. No EC for chisel packer was found in the mentioned literatures. The experimental EC for this machine from table 1, was 14.06 l/ha. This implement needs more power and therefore more fuel use than a chisel alone. Regarding this matter, the EC for chisel packer obtained in the experiment compares well. The EC for other machines seems to be very well adapted comparing to the international figures (Anonymous, 2007; Anonymous, 2007; Cromwell; 1995; Anonymous, 2001; Griffth et al, 2005 and Molenhuis, 2001) in table 3 specially when considering the relatively lower experimental field efficiency in Iran. Significant differences were observed at 1% level between all the machines except for the two implements that is Chisel packer and Chisel ploug.

Table1. Mean data collected and calculations

	Moldboard plough	Chisel packer	chisel	Disk	Mechanical planter	Pneumatic planter
Plowing/Planting time(h/ha)	1.97	1.09	0.85	0.49	0.74	0.76
Turning time (h/ha)	0.69	0.35	0.29	0.22	0.25	0.40
Field efficiency*	73.16[a]	75.33[a]	73.50[a]	68.16[b]	73.80[a]	64.40[c]
Slippage (%)	23.0	12.0	7.7	19.0	14.28	18.81
Speed across field (km/h)	4.57	4.43	5.97	8.09	8.07	7.85
Energy coefficient for plowing/planting (l/ha)*	26.36[a]	14.06[b]	12.19[b]	6.64[c]	4.96[d]	7.10[c]
Energy coefficient for turning (l/ha)	5.41	1.90	2.07	1.04	1.20	1.93
Machine width(m)	1.2	2	2	2.5	1.8	1.8

* Means in rows with different letter are significant at 1% probability level.

Table 2. Comparison between the experimental and the cited FE.

References	Experimental	ASAE	Hunt	Hanna
Moldboard plough	74	85	88-74	85
Chisel plough	74	85	90-75	85
Tandem disk	68	80	90-77	83
Roller packer	74	85	-	-
Row crop planter	74	65	78-55	-
Pneumatic R C planter	63	-	-	70

Table 3. Energy coefficient for machine operations cited from published literatures, l/ha.

References	Moldboard plough	Chisel plough	Tandem disk	Row crop planter	Air seeder
Anonymous (2007)	18	-	12	-	-
Cromwell et al (1995)	17.5	-	4.4	-	-
Cromwell et al (1995)	13.5	-	4.8	-	-
Anonymous (2001)	16.5	10.7	6.8	-	6.3
Griffith et al (2005)	18.0	12.0	-	-	-
Molenhuis (2001)	11.30	5.25	3.75	3.0	-
Anonymous (2007)	16.4	12.6	6.8	4.8	-

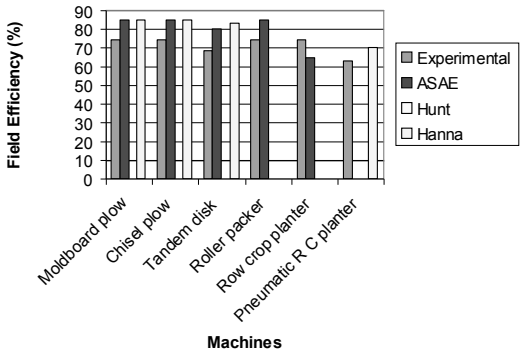

Figure 1. Comparison of the experimental field efficiency and the cited values.

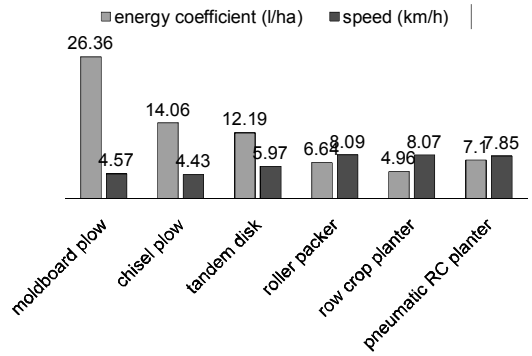

Figure 2. Energy coefficients at the speeds for different machines.

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Effect of Forward Speed, Load and Cabin on Tractor Noise and the Health of Drivers

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Abstract: Noise has caused hearing problems to many drivers in the world. It has been investigated that 30 percent of Sweden's farmers suffer from hearing loss. Similar results to those from Sweden were found in a study conducted by University of Iowa in the United States, indicating that American farm workers are faced with the same noise problems in their daily work. A MF399 tractor without cabin and a Valtra 170 with open cabin were tested for noise level at the driver as well as the bystander ear for no load and loaded cases. Comparison were made between noise level in different forward speeds for each tractor with no cabin and open window cabin on driver's ear and bystander for no load and loaded cases. Also Comparison between no cabin and open cabin in each forward speed for no load and loaded tractor at driver's ear as well as at bystander. The noise level at 2.9 km/h for no load open cabin as well as for loaded no cabin and open cabin at driver's ear was significantly greatest among the related speed ranges. It looked like the 2.9 km/h speed with one exception was a threshold after which the SPL (Sound Pressure Level) dropped. For no load no cabin case at the driver ear with a mean value of 87.72 dB(A) no significant difference was observed at 1% level for speeds up to 3.7 km/h; but the SPL for 6.1 km/h was significantly higher. The permissible exposure time was calculated as 4 h/day and 1.9 h/day respectively. For no load open cabin case no significant difference in SPL between SPL at speeds 1.9, 2.2 and 6.1 km/h with a mean value 83.1 dB(A). However significant difference was observed between 2.9 km/h and 3.7 km/h speed. For loaded no cabin, no significant difference was observed between SPL of 1.9, 2.9 and 3.7 km/h with mean value 93.5 dB(A) and 1.1 h/day permissible exposure time. For loaded open cabin. No significant difference between SPL for 3.7 and 6.1 km/h with mean 89.2 dB(A) and permissible exposure time of 5.4 h/day. Also no significant difference between the SPL for 1.9 and 2.2 km/h with mean 83.7 dB(A). Bar graph for all exposure times are shown. The same comparisons were made at bystander's ear and between no cabin and open cabin in each forward speed.
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Keyword: sound level, noise measurements, tractors, cabin, driver's ear

1. Introduction

Noise or unwanted sound is one of the major sources of discomfort to the workers which affects human both psychologically and physically. It has caused hearing problems to many drivers in the world. Noise is measured in two ways for OECD tests (Anonymous, 2010): at the operator's ear and from a bystander position, and is measured in decibels [dB(A)]. But to the human ear, a noise that sounds twice (or half) as loud is actually measured at 10 dB(A). Sound measurement is made on the test track in two locations-at the driver's ear and in a location representing "bystander noise. The tests at the driver's ear are performed in several gears and under a number of conditions; but only the maximum level is reported. The "bystander sound" test is performed with the microphone located at 7.5 m from the centerline of the tractor which is accelerating from a lower speed to full speed in its top gear. The OECD procedure differs. The SAE/ASABE procedure measures sound in only one gear under different load conditions, whereas the OECD procedure measures sound in different gears between High Idle and Rated Engine speed (Larsen, 2002). Sound levels are recorded using the "A" scale in the sound-level meter and are expressed in terms of decibels (A) or dB(A). The A scale is a filter that responds like a human ear. A 3-dB(A) increase in sound level doubles the sound-pressure level. Therefore, for every 5-dB(A) increase in sound level, the permissible exposure time is cut in half. In other words, at 95 dB(A), the allowable exposure time is only four hours. It is not uncommon to have tractor sound-level reaching 95 dB(A). (Grisso et al., 2007)

Tractor driver farmer had more often high frequency hearing loss when compared to non tractor driver farmer. The noise levels observed on tractors in different operations were in the range of 90–110 dB (A) (Kumar et al., 2005). Sound levels that cause hearing loss begin at about 85 dB(A). Hearing loss occurs more quickly with louder noise. OSHA Standards consider sound measured at 85 decibels or higher as damaging to the eardrum and therefore a risk to hearing (Anonymous, 2004). It has been investigated (Anonymous 2010) that 30 percent of Sweden's farmers suffer from hearing loss. Similar results to those from Sweden were found in a study conducted by University of Iowa in the United States, indicating that American farm workers are faced with the same noise problems in their daily work. An investigation by Dewangan et al (2005) for determination of SPL on 18.7 and 26.1 kW tractors and 4.6 and 6.7 kW hand tractors during field operations with various implements, revealed that both tractors produced the noise of 92 dB(A) in the working zone of operator. The SPL of the hand tractor was about 2 dB(A) higher than that of the tractor. The SPL during field operations at operator ear level increased with increase in engine speed and forward speed.

Celen and Arm (2003)found that the maximum SLP of 97.1 dB(A) was in exhaust pipe and the minimum of 79.7 dB(A) at the bystander ear. An increase of 3 dB(A) was measured for engine speed changes from 1000 to 2000 rpm. Durgut and Celen (2004) measured an 96.6 dB(A) at the drivers ear but a minimum of 67.7 dB(A) for the surrounding. They also found a 6 dB(A) difference when engine speed changed from 1000 to 2000 rpm. According to Aybek et al (2010), statistical analyses showed that type of operation, type of cabins, and operation x cabin interactions were statistically significant at ($P < 0.01$). The use of original cabins had a greater effect in decreasing average sound pressures and resulted in more efficient noise insulation, especially at higher center frequencies compared to field installed cabins. Sound pressure levels at 4000 Hz center frequency was reduced 2–13 dB and 4–18 dB by using a field-installed cabin and an original cabin, respectively. It was concluded that depending on the cabin types used, the operators could usually work from 4 to 6 h a day without suffering from noise induced inconveniences while 2–3 h is permissible for plowing and forage harvesting on tractors without cabins. In reference Anonymous (2009) it is stated that No Member State may refuse to grant EC (European Council) type-approval or national type-approval of any type of tractor on grounds relating to the driver-perceived noise level if that level is within the following limits: 90 dB(A) in accordance with Annex I, or 86 dB(A) in accordance with Annex II. Individual tasks which exceeded 85 dB(A) TWA-8 (NIOSH) identified by researchers were Tilling/plowing, Planting, other farm activities (Milz, 2006)

Equation (1) is given for safe exposure time to noise,

$$t = \frac{8}{2^{\frac{SPL-85}{3}}} \quad (1)$$

(Anonymous, 1996) where t= hours of exposure per day. An experiment in Croatia by Goglia and et al (2005) showed that by ISO 4872, 6393 and 362 standards, the noise level did not exceed the limit values. However, the noise level at the operator's position at full load and at nominal load exceeded the limits. Noise levels of 155 tractors on 36 farms were studied (Holt et al., 2006) The range of noise levels at the driver's ear with radios off and windows closed (if so equipped) was from 78 to 103 dB(A). Seventy-five percent of tractors without cabs had noise levels in excess of 90 dB, compared to only 18% of tractors with cabs. The use of a radio adds an average of 3.1 dB of noise. A specially selected group of 45 farm tractor drivers were examined in order to estimate the degree of occupational hearing loss (Holt et al., 2006). The drivers, aged 21-50 years, were employed on multi production farms. The study showed that the operators under study had statistically worse hearing within the range of high frequencies (3-6 kHz), especially those aged over 30 years. A study was carried out when a fabricated cab was added to an agricultural tractor (Abd-el-Tawwab et al., 2000). The fabricated cab was selected after a comprehensive series of experimental tests carried out on a variety of cab constructions. The results were discussed from the view point of obtaining the influence of the tractor driving parameters (road speed, gear-shift, engine speed and tractive effort) on the noise measured inside the tractor cab and over the frequency range up to 2000 Hz. In April, however, the occurrence of high total exposure values was due to intensive field activities (plowing, harrowing, sowing), and prolonged exposure to this factor (Aybek et al., 2010). In the seasons of the year analyzed, high equivalent exposure values were observed within the range: 5.53-6.61 Pa^2 h (some polish standards). Mean value for this parameter for the whole year reached the value of 4.27 Pa^2 h (standard exceeded 4.3 times). This value is equivalent to a mean exposure level equal to 91.3 dB.

2. Material and Methods

Two types of tractor a 2-wheel drive MF399 with 62 kW PTO power without cabin and a Valtra T170, 184 kW power with cabin were tested. No load and loaded with mouldboard plough were tested for

noise level at different forward speed. The measurements were taken at the driver ear and at a distance 7.5m from centre axis of tractor according to OECD standards(Anonymous 2010). A sound meter of type Lutron SL4013 equipped with capacitance microphone was used. The test course was a plot of 100 m long by 40m wide in open field. Each experiment data was recorded with 9 replicates. Data was analyzed based on factorial experiment with MSTAT-C software.

3. Results

The results are shown in Graphs 1-6 and tables 1-2. Comparison results were as follows:

1. Comparison between noise level in different forward speeds for each tractor with no cabin and open window cabin on driver's ear and bystander for no load and loaded cases

1.1. At driver's ear.

1.1.1. The noise level at 2.9 km/h for no load open cabin (graph 2), loaded no cabin (graph 3) and open cabin (graph 4) was significantly greatest among the related speed ranges as shown in Graph 1 and in third row in table 1. It looks like the 2.9 km/h speed is a threshold after which the SPL (Sound Pressure Level) drops although exception is seen in graph 3. Also although the trend is true for graph 1 but the SPL rises for 6.1 km/h speed. The permissible exposure time were 1.9, 2.5, 0.9 and 1.9 h/day for graphs 1, 2, 3 and 4 respectively as is shown in Graph 5 and in last row in table 1.

1.1.2. No load no cabin case. With a mean value of 87.72 dB(A) no significant difference was observed at 1% level for speeds up to 3.7 km/h; but the SPL for 6.1 km/h was significantly higher. The permissible exposure time was 4 h/day and 1.9 h/day respectively.

1.1.3. No load open cabin case. No significant difference in SPL at speeds 1.9, 2.2 and 6.1 km/h with a mean value 83.1 dB(A). However, significant difference was observed between 2.9 km/h and 3.7 km/h speed. The calculated exposure time for the latter speed was 6 h/day.

1.1.4. Loaded no cabin. No significant difference between SPL of 1.9, 2.9 and 3.7 km/h with mean value 93.5 dB(A) and 1.1 h/day allowed exposure time. Also no significant difference between 2.2 and 6.1 km/h but; a permissible exposure time about 6.3 h/day.

1.1.5. Loaded open cabin. No significant difference between SPL for 3.7 and 6.1 km/h with mean 89.2 dB(A) and permissible exposure time of 5.4 h/day. Also no significant difference between the SPL for 1.9 and 2.2 km/h with mean 83.7 dB(A). Bar graph for all exposure times are shown in Graph 5.

1.2. At bystander's ear.

Results for this case are shown in Graph 2 and columns 4,5,7 and 8 of table1. The graphs 1-4 in Graph 2 shows ups and downs but all SPL are lower than the safe level, 85 dB(A) which is set by NIOSH standards. Therefore, there is no limitation on the bystander's exposure time. For open cabin case, no load and loaded (graphs 2 and 4), the sound level increases up to speed 2.9 km/h but decreases after ward which is the same pattern for the same case at the driver's ear. For no cabin, no load (graph 1), the SPL is almost increasing but up to 3.7 km/h speed which does not resemble with the one at the driver's ear. However, the graph 3 follows the same pattern although with a wide gap with respect to the no load case. Graph 4 shows the bar graph for these cases. Allowable exposure time is shown in Graph 6.

2. Comparison between no cabin and open cabin in each forward speed for no load and loaded at driver's ear as well as at bystander. With reference to table 2:

2.1. At 1.9 km/h forward speed.

2.1.1. There was significant difference between the no cabin and open cabin noise level at the driver's ear (Table 2). Considering the doubling noise for a difference of 3 dB(A) [12], the sound level increases more than double at the driver's ear for no cabin loaded tractor compared to open cabin tractor (fourth column in table2). SPL for all cases was lower for open cabin compared to no cabin.

2.1.2. Significant difference was observed for loaded tractors at the bystander ear but; not so for the no load case.

2.2. At 2.2 km/h forward speed.

Significant difference observed between the SPL for no cabin and open cabin at the driver's ear for no load and loaded tractors. Data for open window were lower than that for no cabin. The SPL at the bystander's ear were not significant in either cases.

2.3. At 2.9 km/h forward speed.

SPL differences were significant for only the no load case at the bystander ear.

2.4. At 3.7 km/h forward speed.

Significant difference for the no load case at the bystander ear and for loaded tractors at the driver ear.

2.5. At 6.1 km/h forward speed.

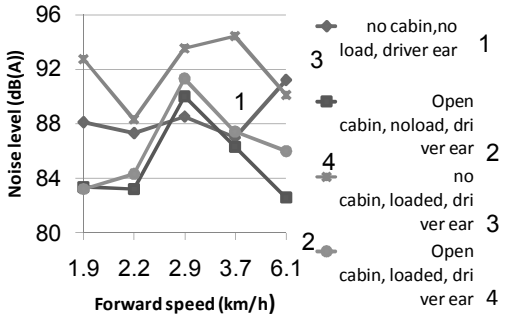
No significant difference for only the loaded case at the bystander ear.

4. Discussion

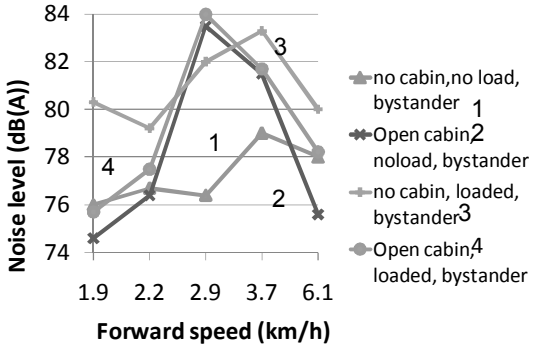
For no cabin and open cabin both, data showed an increasing SPL with increase in forward speed up to 2.9 km/h as it was observed by Dewangan et al (2005) but; it decreased afterwards. One exception was for the no cabin loaded tractor at the driver's ear that the SPL continued rising up to 3.7

km/h and then dropped. The tractors were up shifted from Low 3 to Hi 1 to increase the speed from 3.7 km/h to 6.1 km/h. This shifting could have caused the drop after 3.7 km/h because of decreased torque and relieving the engine. No reason can be thought of for justifying the SPL drop after the 2.7 km/h. More research on this matter is recommended. Another exception was observed for no cabin, no load case at the driver's ear in which the sound level increased after the 3.7 km/h to 6.1 km/h. This may be attributed to inaccuracy in data because it contradicts the results for the other three cases. For the usual ploughing speed of 4.5 km/h to 5, in Iran, the driver, as calculated from equation (1) for 92 dB(A), should not be driving for more than 1.5 hours a day unless putting on some kind of ear protections. The result somehow agrees with Aybek and et al (2010) who allow ploughing for 2-3 hours a day. The maximum data point did not get over 96 dB(A) while Durgut and Celen (2004) reported it as 96.6. However, the noise level was in fact over 85 dB(A) for field operation as said by Solecki (2010). Holt et al (2006) obtained a value of 78 to 103 dB at the driver ear for tractors with closed cabin and with radio off while in this research, with even open cabin, the SPL did not reach 91 dB(A). Celen and Arm (2003) reported a minimum of 79.7 dB(A) noise level at bystander ear which compares well only for the no cabin, loaded case. The minimum here was less than 76 dB(A) for other cases with or without cabin. This might be due to the better make of engines with new technology.

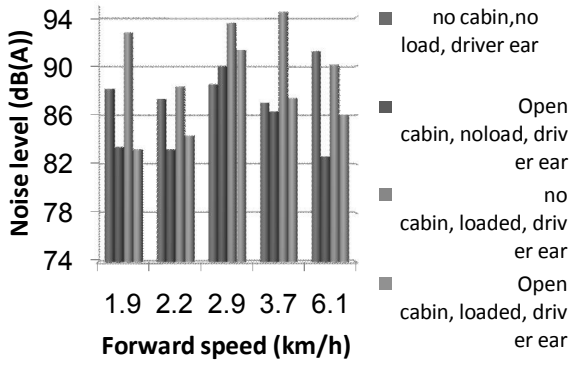
Finally, both the load and speed affects the sound level. While higher speeds over 4 km/h decreases the SPL, higher load on the engine increases it. For usual field operation, no harm at the bystander's ear but the driver at low speeds should either limit the exposure hours or wear some kind of ear protection while driving on no cabin tractor or even with cabin tractor if he is to frequently open the windows. Engines with new technology help in reducing noise.



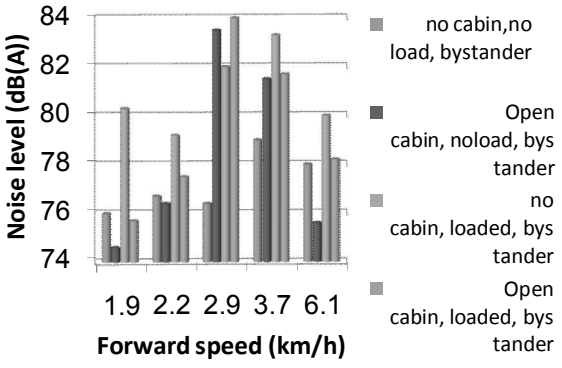
Graph 1: Mean noise level for different forward speeds, at driver ear.



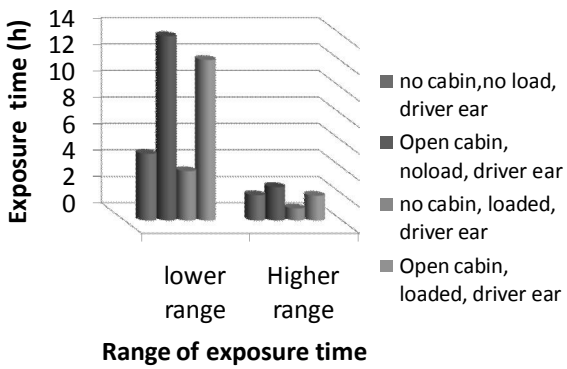
Graph 2: Mean noise level for different forward speeds, at bystander ear.



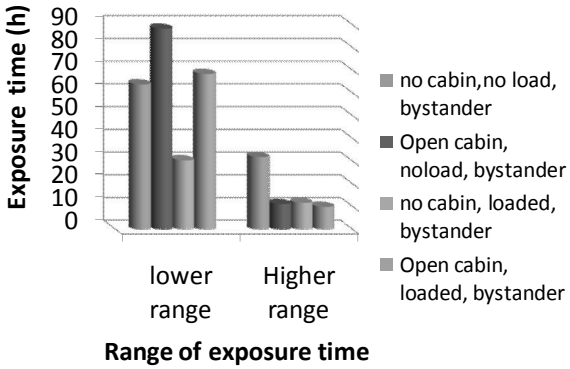
Graph 3: comparison between no cabin and open cabin noise level at driver's ear.



Graph 4: comparison between no cabin and open cabin noise level at bystander's ear.



Graph 5. Allowable exposure time for driver's ear.



Graph 6. Allowable exposure time for bystander's ear.

Table 1. Comparison of noise level in different forward speeds for each tractor with no cabin and open window cabin on driver's ear and bystander for no load and loaded cases.*

Forward speed km/h	Driver ear No load		Bystander ear No load		Driver ear loaded		Bystander ear loaded	
	No cabin	Open cabin	No cabin	Open cabin	No cabin	Open cabin	No cabin	Open cabin
1.9	88.1^{b}	83.4^{c}	76.0^{a}	74.6^{b}	92.7^{a}	83.2^{c}	80.3^{b}	75.7^{c}
2.2	87.3^{b}	83.2^{c}	76.7^{a}	76.4^{b}	88.3^{b}	84.3^{c}	79.2^{b}	77.5^{c}
2.9	88.5^{b}	90.0^{a}	76.4^{a}	83.5^{a}	93.5^{a}	91.3^{a}	82.0^{a}	84.0^{a}
3.7	87.0^{b}	86.3^{b}	79.0^{a}	81.5^{a}	94.4^{a}	87.4^{b}	83.3^{a}	81.7^{b}
6.1	91.2^{a}	82.6^{c}	78.0^{a}	75.6^{b}	90.1^{b}	86.0^{b}	80.0^{b}	78.2^{c}
Min.	87.00	82.60	76.00	74.60	88.30	83.20	79.20	75.70
Max.	91.20	90.00	79.00	83.50	94.40	91.30	83.30	84.00
Mean	88.42	85.1	77.22	78.32	91.8	86.44	80.96	79.42
SD	1.67	3.09	1.25	3.93	2.53	3.15	1.66	3.36
Lower exposure(h)	5.04	13.93	64.00	88.44	3.73	12.13	30.55	68.59
Higher exposure(h)	1.91	2.52	32.00	11.31	0.91	1.87	11.85	10.08

* Means with different letters at different forward speeds in each column are significantly different at 1% probability level.

Table 2. Comparison between no cabin and open cabin in each forward speed for no load and loaded at driver's ear as well as at bystander.*

Forward speed km/h	Driver ear No load		Bystander ear No load		Driver ear loaded		Bystander ear loaded	
	No cabin	Open cabin	No cabin	Open cabin	No cabin	Open cabin	No cabin	Open cabin
1.9	88.1^{a}	83.4^{b}	76.0^{a}	74.6^{a}	92.7^{a}	83.2^{b}	80.3^{a}	75.7^{b}
2.2	87.3^{a}	83.2^{b}	76.7^{a}	76.4^{a}	88.3^{a}	84.3^{b}	79.2^{a}	77.5^{a}
2.9	88.5^{a}	90.0^{a}	76.4^{b}	83.5^{a}	93.5^{a}	91.3^{a}	82.0^{a}	84.0^{a}
3.7	87.0^{a}	86.3^{a}	79.0^{b}	81.5^{a}	94.4^{a}	87.4^{b}	83.3^{a}	81.7^{a}
6.1	91.2^{a}	82.6^{b}	78.0^{a}	75.6^{b}	90.1^{a}	86.0^{b}	80.0^{a}	78.2^{a}

* Means with different letters at different loading and driver ear as well as bystander in each forward speed are significantly different at 1% probability level.

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The influence of stitch length of weft knitted fabrics on the sewability

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Abstract: The sewing needle penetration force, the feed of the sewing material, sewing needle size are very important parameters during the sewing of knitted fabric. This paper is studying the effect stitch length, yarn count, and needle size on the penetration force which indicates the fabric sewability. Some physical and mechanical properties of these fabrics were also tested. From the results of this study obtained, we can conclude that, the sewability (which is indicated by penetration force) of 100% cotton single jersey fabric is inversely proportional to its stitch length. In the same time the fabric with coarser yarn count gives the higher penetration force. Also we can conclude that, fabrics with coarser yarn counts and shorter stitch length have better functional performance characteristics.
[Z. M. Abdel Megeid, M. Al-bakry, and M. Ezzat, The influence of stitch length of weft knitted fabrics on the sewability. Journal of American Science 2011;7(8):610-617]. (ISSN: 1545-1003). http://www.americanscience.org.

Key Words: stitch length-weft knitted fabric-sewability- abrasion resistance- Air Permeability

1. Introduction:

Knit fabrics are having wider use in time since they can be produced more easily for a lower cost, and they are more flexible .However, knit fabrics are less than woven fabrics since they are produced with low twist yarn and have a slack construction, as a result of which they have a low abrasion resistance.

Abrasion resistance and pilling performance are two of the most important mechanical characteristics of fabric. The resistance of a fabric against the force of friction is known as the abrasion resistance. [1]

There are many factors, such as yarn spinning system, fabric construction and finishing processes that affect in the abrasion resistance. With certain precautions taken in fabric production, the abrasion resistance of knit fabrics can be developed positively. [1]

In the case of weft knitting, the dimensional properties of staple weft knitted fabrics depend mainly on the average loop length. In an earlier work, many attempts were made by researchers to analyze the relationships among the dimensions of knitted fabrics, the properties of the constituent yarns and the variable factors in knitting. It was noticed that the dimensional and weight related properties of knitted in relaxed state were determined uniquely by length of yarn in the stitch [2].

T. Ogulata and S. Mavruz have shown that the fabric with the lowest course count per cm and yarn count in Tex has the highest air permeability values. Moreover, increasing the loop length produced a looser surface in the fabric and decreased air permeability. As the yarn gets thinner and the pores between loops gets larger, the air permeability will increase accordingly. According to some formulations, when the stitch density, stitch length or yarn diameter increase, pore size values decreases. [3].

The sewing needle penetration force, sewing thread tension the feed of the sewing material, as well as selection of the sewing thread, sewing needle size and shape of the sewing needle point are very important parameters during the sewing of knitted fabric.

Sewing thread plays an influential role in seam design; it should be stretchable since knitted fabric has elastic properties in the direction of courses and Wales .The size of the needle eyelet and thread thickness should be mutually adjusted in order for the thread to pass through the eyelet with as little friction as possible.

The quality of a sewn seam depends on these two factors, therefore it is necessary to match the thickness and density of the knitted fabric with the fineness of sewing thread and needle.

The penetration force of a sewing needle is mostly based on the friction occurring between the sewing work pieces and sewing needle, and the highest penetration force of the sewing needle occurs at the moment when the sewing needle penetrates the sewing material, which should be taken into consideration, especially with knitted fabrics.(4)

The sewing needle penetration force is one of the most significant technical parameters in the sewing process affected by various factors such as: type, number of layers of the sewing material, and needle size [4].

The stitch length for each fabric was calculated as the ratio of course length to the number of needles [5].

Doyle and Hurd (1953) found that the stitch density of plain knitted fabrics in the dry relaxed state is dependent only on the loop length, and independent of other yarn and knitting variables [6].

This paper is studying the effect of stitch length, yarn count, and needle size on the penetration force which indicates the fabric sewability

2- Experimental work:

1-The knitted fabric samples were made of 100% cotton.

Produced 9 sample fabrics from single jersey, machine (circular knitting) gouge 24 Diameter of cylinder 26/inch by using combed cotton with 3 English counts 20/1, 24/1, 30/1 at different stitch length (1.3 -1.8 -2.7mm) and rate of feeding 135, 155, 175

- The physical properties test:

- Fabric weight/g square meter according to ASTM D3776
- Air permeability/ $Cm^3/cm^2/sec$ according to ASTM D 737-96
-Thickness/ mm according to ASTM according to ASTM D1777-96
-stitch length using Huttra course length tester according to ASTM D 3887.

The mechanical properties test:

The L&M sew ability tester:

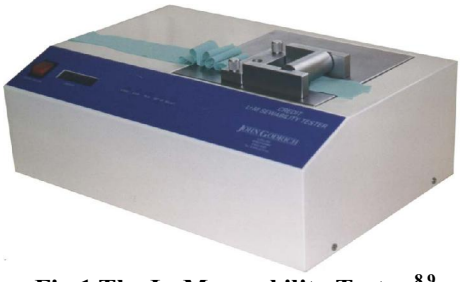

Fig 1 The L+M sewability Tester [8,9]

Testing fabric sewing properties, US patent 3979951, 1976), a device used in many studies on needle penetration force. This equipment simulates a sewing machine by penetrating the tested fabric with an unthreaded needle, at a rate of 100 penetrations per min., with needle count 70, 80.

-Abrasion resistance / cycle according to ASTM D 4966

-Burst strength/ Kg/cm^2 according to ASTM D 3786-01

The physical and mechanical properties of these fabrics were tested under standard condition.

3-Result and discussion:

The results of tested samples are shown in table (1).

Table (1) The Average results for Tested Samples

S	Ne	S.L/ mm	Sewability/N		Thickness/ mm	Air permeability / $Cm^3/cm^2/sec$	Abrasion / cycle	Weight/g	Burst Kg/cm^2
			N70	N80					
1	20	1.3	38	55	58	23.8	410.0	240.4	11.00
2	20	1.8	36	26	62	54.3	350.0	212.4	9.20
3	20	2.7	18	12	63	112.0	300.0	207.1	9.00
4	24	1.3	32	27	56	52.90	290.0	199.7	8.40
5	24	1.8	26	14	60	107.9	175.0	177.5	7.70
6	24	2.7	8	7	54	118.4	193.3	155.0	6.40
7	30	1.3	7	11	59	126.6	123.3	150.6	7.70
8	30	1.8	6	8	89	136.7	163.3	128.2	6.30
9	30	2.7	6	5	79	272.6	124.0	118.4	5.93

S : Sample number
Ne : Count 20 24 30
S.L : stitch length
Sew ability :Sewing needle penetration force/N
N : needle number 70-80

3.1. Effect of stitch length on Sewability:

a. Sewing needle penetration force; Sewability (for needle number N70):

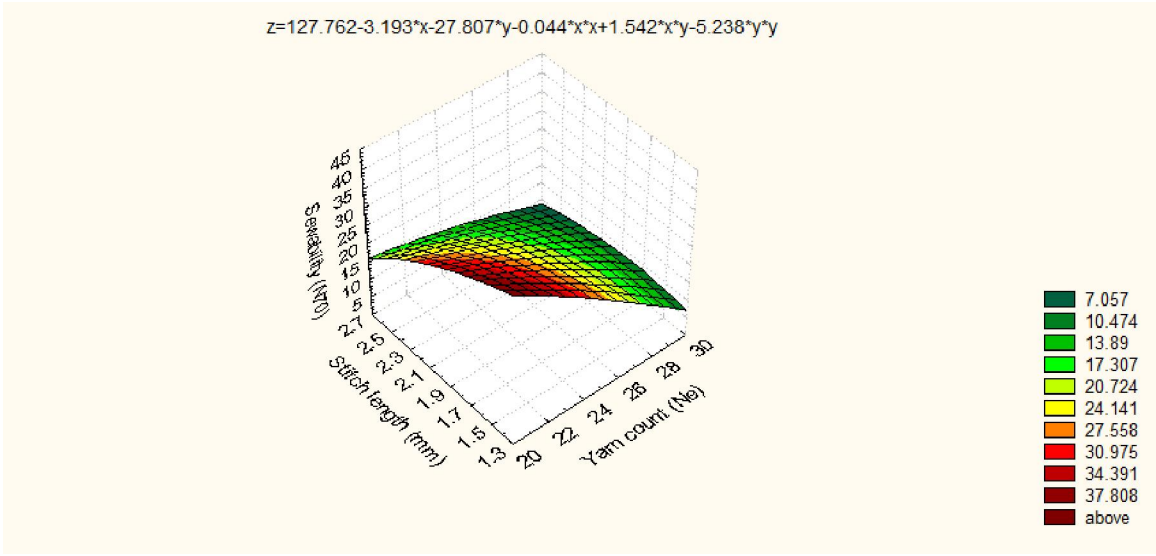

Fig (1) The Relation between Stitch Length and Sew ability (N70) for Different Yarn Counts

From figure (1) it is observed the relation between stitch length and sewability by using (Needle 70) that stitch length of 100% cotton single jersey fabric is inversely proportional to its sewability. In the same time the fabric with coarser yarn count gives the higher penetration force and vise versa. This result may be explained by the fabric thickness and tightness factor which are decreased with the finer count and longer stitch length which allows the needle to penetrate easily.

b. Sewing needle penetration force; Sewability (for needle number N80):

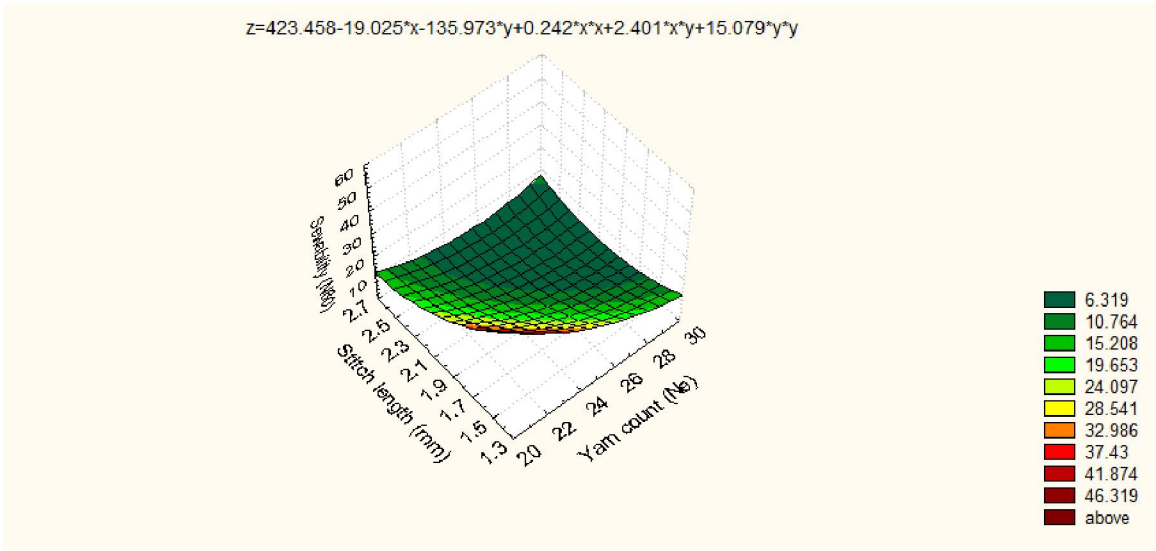

Fig (2) The Relation between Stitch Length and Sew ability (N80) for Different Yarn Counts

3.2. Effect of stitch length on some fabric Properties:

a. Fabric thickness:

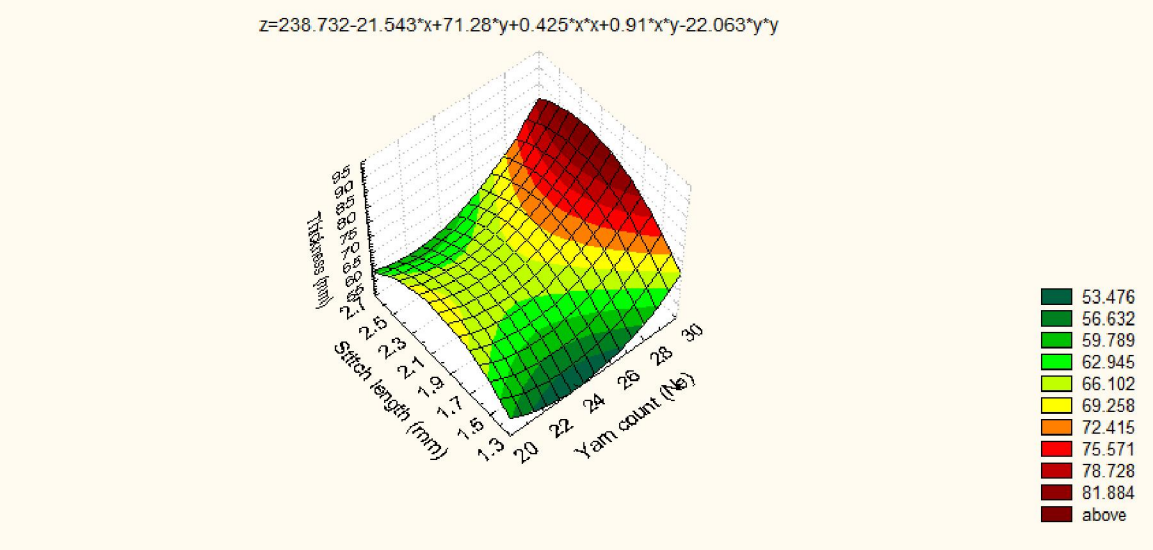

Fig (3) the Relation between Stitch Length and Fabric Thickness for Different Yarn Counts

Figure (3) shows the relation between stitch lengths and the fabric thickness, which indicates that stitch length, is inversely proportional to its measured thickness. In the same time the fabric with coarser yarn count gives the higher fabric thickness and vise versa.

b. Fabric weight:

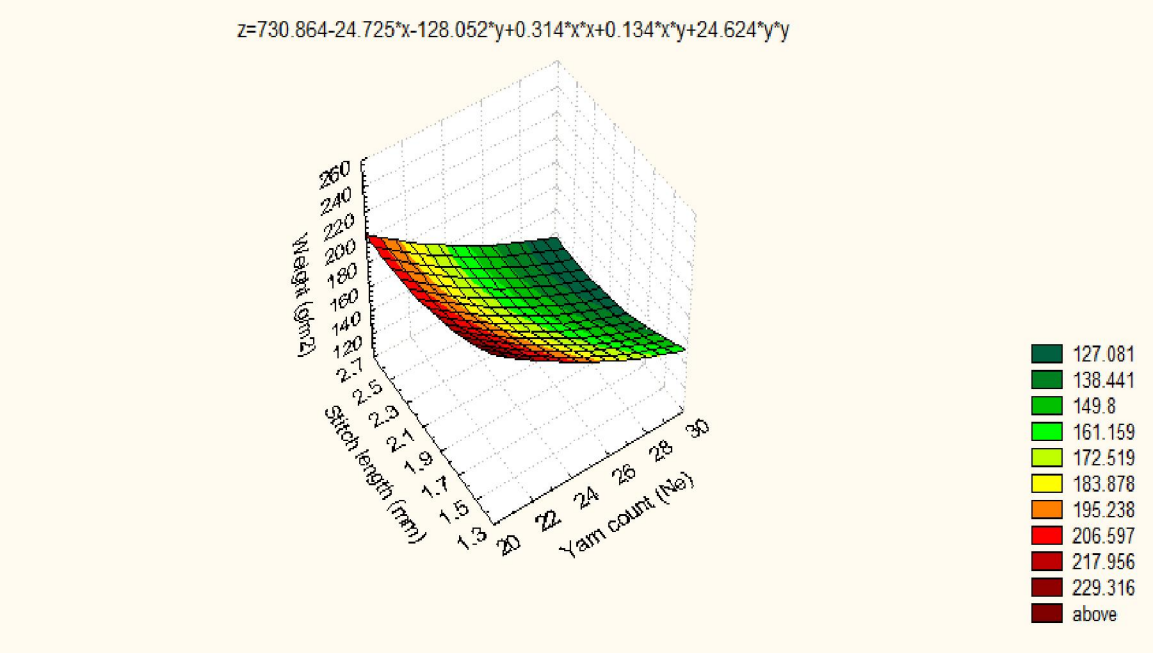

Fig (4) the Relation between Stitch Length and Fabric Weight for Different Yarn Counts

Figure (4) shows the relation between stitch lengths and the fabric weight, which indicates that stitch length, is inversely proportional to its measured weight. In the same time the fabric with coarser yarn count gives the higher fabric weight and vise versa.

a. Fabric Air Permeability:

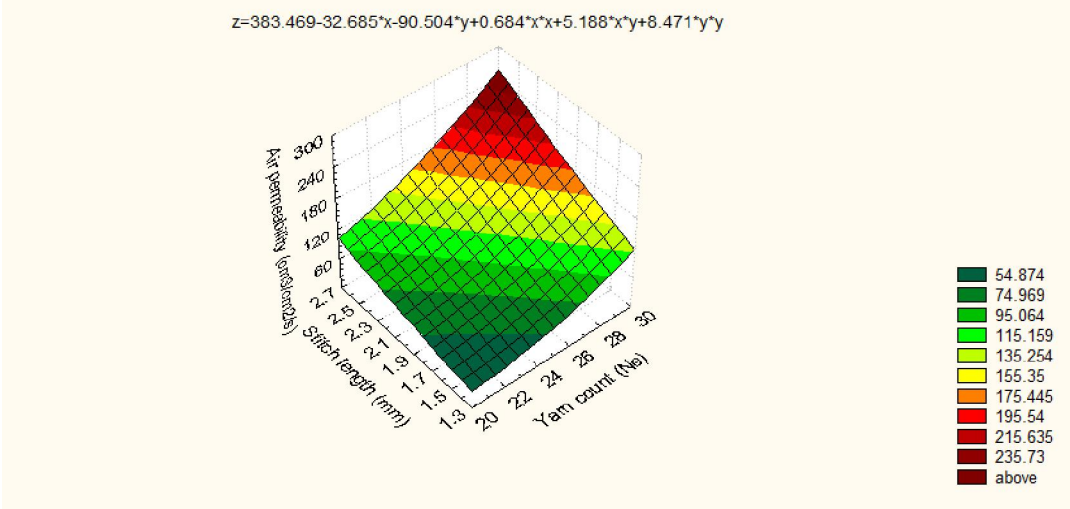

Fig (5) the Relation between Stitch Length and Air Permeability for Different Yarn Counts

Figure (5) shows the relation between stitch lengths and the fabric air permeability, which indicates that stitch length, is directly proportional to its measured air permeability. In the same time the fabric with finer yarn count gives the higher fabric air permeability. This result may be explained by the tightness factor which is decreased with the finer count and longer stitch length which allows the air flow to penetrate easily thru the fabric.

b. Fabric abrasion resistance:

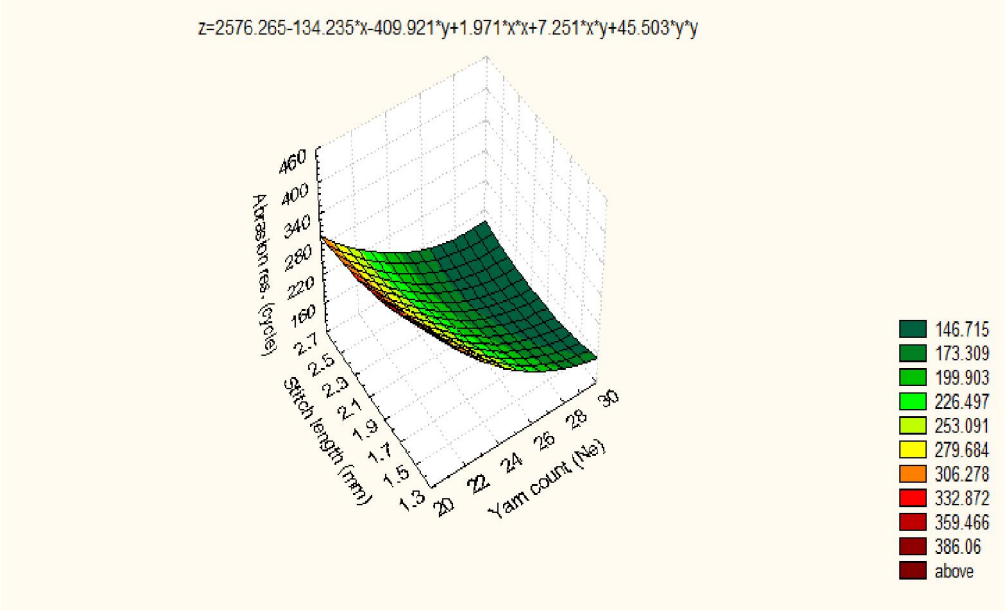

Fig (6) the Relation between Stitch Length and Fabric Abrasion for Different Yarn Counts

Figure (6) shows the relation between stitch lengths and the fabric abrasion resistance, which indicates that stitch length, is inversely proportional to its measured abrasion resistance. In the same time the fabric with coarser yarn count gives the higher fabric abrasion resistance.

c. Fabric bursting strength:

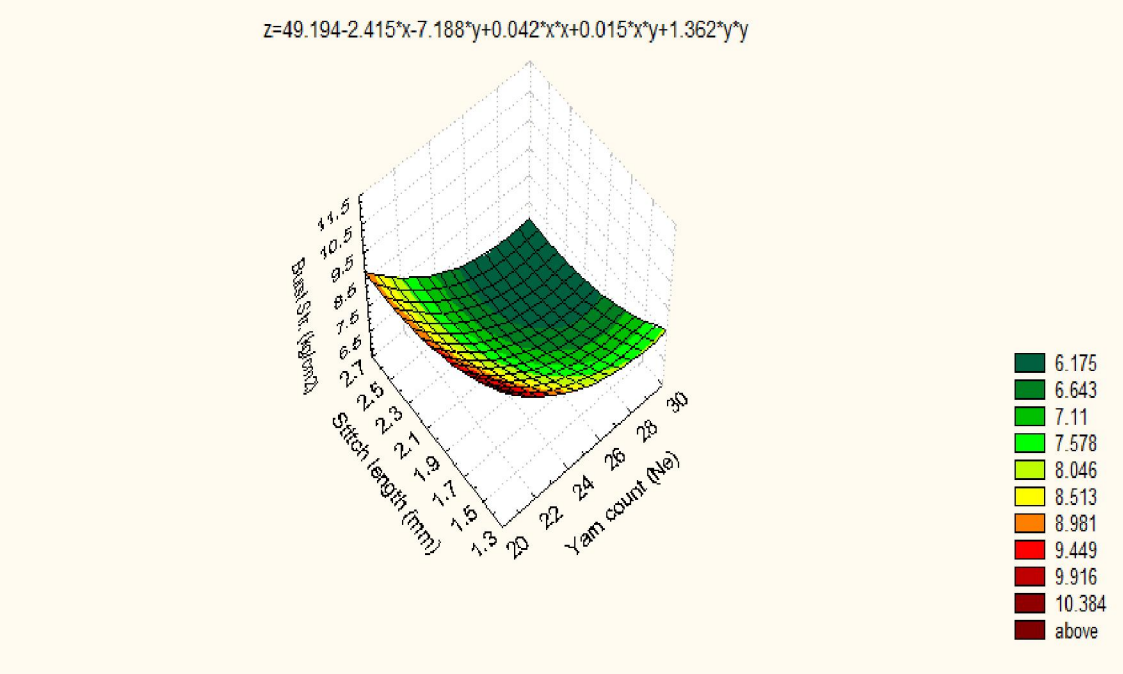

Fig (7) the Relation between Stitch Length and Fabric bursting strength for Different Yarn Counts

Table (2) quality factors of the fabrics under study

	Bursting Strength	Abrasion Strength	Air permeability	Sewability N80	Sewability N70	Quality Factor
Ne 20, SL 1.3mm	100	100	98	55	58	82.2
Ne 20, SL 1.8mm	92	93	71	60	58	74.7
Ne 20, SL 2.7mm	91	87	60	71	67	75.1
Ne 24, SL 1.3mm	88	85	72	59	59	72.8
Ne 24, SL 1.8mm	85	71	61	68	62	69.3
Ne 24, SL 2.7mm	79	74	60	86	88	77.1
Ne 30, SL 1.3mm	85	65	59	73	93	74.9
Ne 30, SL 1.8mm	79	70	58	81	100	77.6
Ne 30, SL 2.7mm	77	65	54	100	100	79.3

Figure (7) shows the relation between stitch lengths and the fabric bursting strength, which indicates that stitch length, is inversely proportional to its measured bursting strength. In the same time the fabric with coarser yarn count gives the higher fabric bursting strength.

3.3. Assessment of fabric quality with different stitch lengths and yarn counts:

An assessment approach to the quality of fabrics under study was carried out to indicate the overall quality level of the fabrics based on the performance characteristics of these fabrics when manufactured as knitwear.

This assessment is done by recalculating the measured values of these quality attributes according to a new dimensionless scale between 0 and 100. This way helps to compare between different samples.

Figure (8) shows radar chart of each fabric, and table (2) shows their quality factors.

From these figures and table we can conclude that, fabrics with coarser yarn counts and shorter stitch length have better functional performance characteristics, while fabrics with finer yarn counts and longer stitch length have better sewability characteristics.

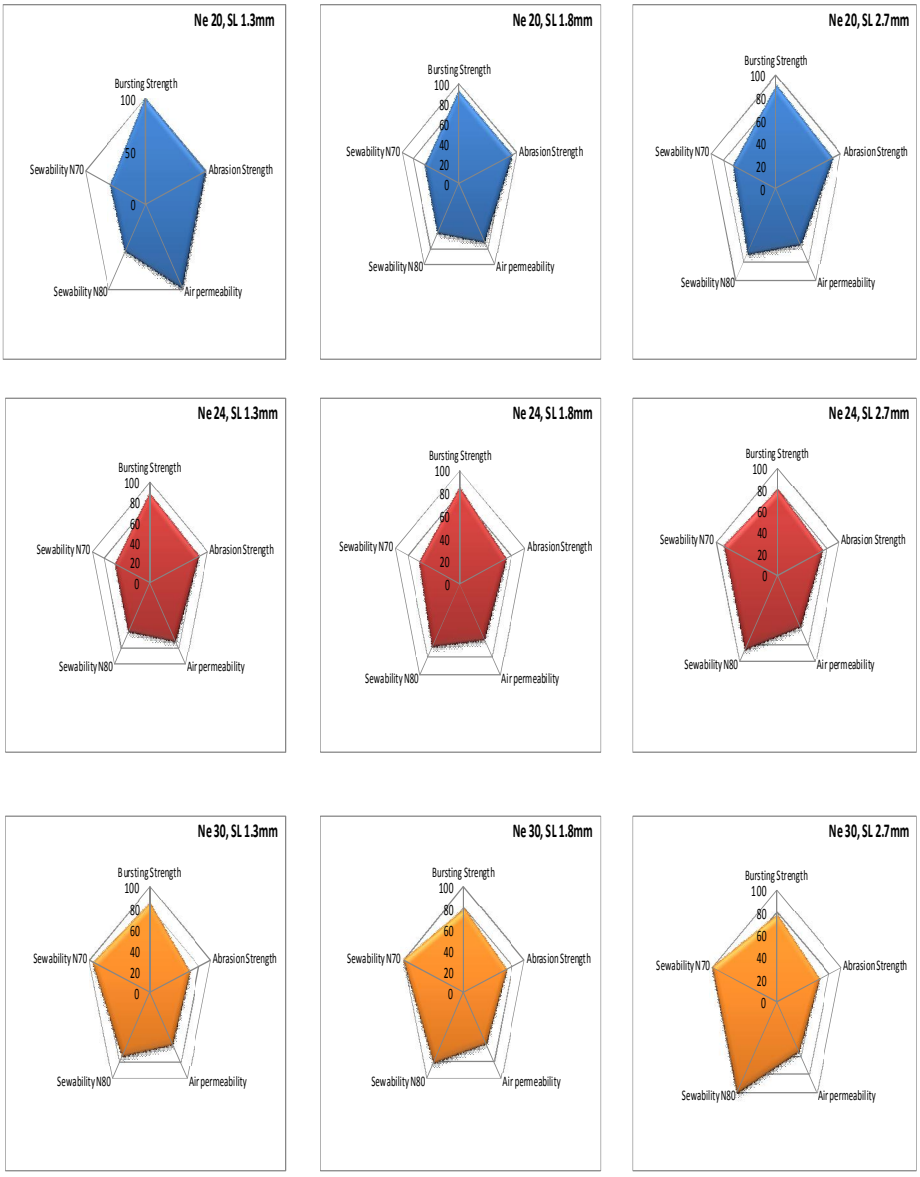

Fig (8) the Radar charts of the nine fabrics with different Stitch Lengths and Yarn Counts

Conclusion:

From results we can conclude that, the sewability (which is indicated by penetration force) of 100% cotton single jersey fabric is inversely proportional to its stitch length. In the same time the fabric with coarser yarn count gives the higher penetration force. Also we can conclude that, fabrics with coarser yarn counts and shorter stitch length have better functional performance characteristics.

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Considering the effect of globalization on ethnic identity of Kurd identity at Sanandaj city in 2010

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Abstract: This research intends to study any effects of globalization on ethnic identity of Kurd identity at Sanandaj City in 2010. The statistical population of this research is all people more than 15 years old and residing at Sanandaj city (223675) in 2010. We appointed 767 persons as the statistical sample and in accordance with Kukran formula. In theoretical basics of this research we have ethnic identity theory and globalization attitude and any effects of globalization on ethnic identity. The general result is a reduction in any dependency and interests in personal ethnic identity by globalization and obtaining the responses. The result of regression analysis shows that globalization indexes and absorption in world nature (Common royalty, Common interests & Social relations preference) may specify about %.531 of tribal nature variance in this function.
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1. Introduction

Although globalization has mainly and naturally an economic situation but in most cases it had converse results as well. It has tried to provide special forms of the culture from clothing up to make up and making similar living methods of human beings from one side and with a form of equalization and in contrast either for motivated reactions and/or naturally various identity on the other. Therefore it is impossible to ignore the element of globalization especially in identity discussions (Apadori, 1996).

Robertson believes globalizations is like compressing the world and change it into a unique place. Globalization means increasing knowledge about the world as a whole with some consequences like formation of identity and ethnic identity as well (Robertson,135:2005). Globalization means publication of specialty and specialization of public. Therefore we have various cultures from one side and cultural unity on the other (Robertson:1992, adapted from Hobbi et. Al, 2010: 104). Cultural specialists intend to show who it is possible for globalization process to compress, expand and deepening time and place for all people around the world and create different conditions for a unique world society (Sklair, 1999:154). In other words, by globalization we have a lot of possibilities for such aspects and effectiveness of it in a wide scope of the world. Such a wide global expansion may create a world identity (Ghoreishi, 2002:43-44). In his work under the title of Globalization & identity identity in Iran, Nassaj has concluded that globalization may cause a type of destruction in long-term and if the culture of these ethnic identities are in lack of different parameters such as world potentials, enriched written heritage, lack of exclusivity in a country and combination with religion (Nassaj 2008L153). A research made by Hobbi Akran et. al shows more increase of global identity among university students there will be a reduction in their local identity. This means that by increasing of globalization process, we will have a reduction in local identities (Hebi et. Al ,2010). But it seems that such a process will occur due to its internal logic and all structural fields and any efforts for maintenance of local dialects – either by governmental authorities and/or sub-cultures- may result only extending such a process and not its stoppage. Regarding all tribal identity elements including to be interest in mother land, depending upon mother language, local customs, to be interested in common names and common living places and also effective factors on tribal identity resistance against globalization, it seems that all local ethnic languages and dialects will be mixed with each other without any resistance by nations and even establishment of provincial T.V. networks and local newspapers. But even it may cause a reduction in its speed of expansion.

Globalization and growth of media may provide this facility for different culture to keep themselves. But whether all people are entitled to benefit from this facility? Wisdom and conceptual selection in long –term may be resulted in preference of most people to learn an official language to their own mother language. Such a combination is possible for different languages such as Turkish and Kurdish for different generations. As a result, the present research has been made to answer this basic

question whether how much is the effect of globalization on identity of Kurd nation in various dimensions?

2. Material and Methods

Regarding the effect of globalization on ethnic & local identities most theorists focused on positive aspect of globalization and the other consider its negative consequences. In other words, those who may believe in positive effects of globalization on ethnic identity believe that globalization will be effective directly or indirectly on ethnic identities. By providing different changes in directions and attitude of different classes and people, globalization may cause a change of direction from social, political subjects into cultural items. The base of future challenges of globalization will be ethnic identity demands. The opponents believe that globalization at major levels of the world may cause a similarity in culture of all nations and unique goals as well.

Research method

Measuring is the used method in this research for collecting required information through questionnaire technique.

Statistical population and sample volume

The statistical population of this research is over 15 year old people residing at Sanandaj city in 2010. Therefore according to the population the number of people with mentioned conditions is 223675 persons in Sanandaj city in 2009. Sample volume in accordance with Kukran formula is 767 persons.

Sampling method

Branch sampling method in this research may guarantee any selection of different sample. The major task is to select sample areas and initially from great areas and then gradually we will have smaller areas. Finally we will select a family sample and use a method for appointing persons in selected families as well.

Factor analysis of World identity

The independent variant of this research is to be absorbed in world identity. Then in order to evaluate this variant we inserted 10 dialects in mentioned questionnaire with which we could provide factor analysis as well. Regarding the following table, the percentage of specified variant is equal to 54.2. Since the special amount of this factor are greater than 1 with factor analysis of all these dialects is close to 1, as a result we may conclude that factor credit of this variant is in compliance with acceptance relevant theories. Furthermore the results of both Karizer & Bartlet tests reveal this reality that it is better to have factor analysis for the mentioned dialects.

Since the special amounts of these factors are greater than 1 with close factor load to 1, therefore we may conclude that factor credit of this variant is suitable with accepting all relevant theories. (Table1)

Table1: The results of factor analysis of different speeches of ethnic identity

Factor	Title of factors	Speeches	Factor load	Determined variance	Special amounts
Global identity	Common loyalty	-All around the world is my land	0.74	24.6	4.2
		-In spite of language, religion and race, all people are respectful.	0.73		
		-I believe in this idea that "All people are like the organs of one body"	0.71		
		-I am responsible against a person only as a person like me.	0.64		
	Common interests	-I am interested in humanistic activities around the world.	0.52	19.5	3.2
		-We should try to have a calm and free of war world.	0.74		
		-I respect the charter of human rights.	0.74		
	Social relations preferences	-World values (humanistic) are more important for me than national tribal ones.	0.68	10.1	2.9
		-It is better not to confidence in foreigners (Those who are not Iranians)	0.68		
		-I cannot accept to be a friend, colleague or neighbor of a non-Iranian person.	0.69		
KMO=0.761 Bartlett's Test of Shericity: 1413.87 Sig=0.000					

Factor analysis of dependent variation of research

We used 27 speeches in this research. Then exploration factor analysis has been used in order to measure tribal identity and specifying different dimensions of tribal identity and its major variants. Varimax circulation method is the used method in this variant like the same independent one. Regarding the following table, we have special amounts for this

variant both greater than 1 and factor load close to 1. As a result we may conclude that there is enough credit for different speeches and measuring of above-mentioned variants. The results of both Kaizer & Bartlet tests indicate that it is useful to have factor analysis for these speeches.(Table 2&3)

Table 2: The results of factor analysis of different speeches of ethnic identity

Title of factors	Speeches	Factor load	Specified variant	Special amounts
To be interested in mother land	I am in love of my land.	0.75	14.6	3.2
	Certainly it is a great pleasure for a person to be at service of his/her mother land.	0.74		
	I am ready to work in any place except in my homeland.	0.73		
	I am responsible against my job in my homeland.	0.68		
	In case of providing better job facilities, I may not accept to live in any place rather than my homeland.	0.74		
To be interested in mother language	I am in love of my mother language.	0.62	11.5	19.2
	I am proud of Kurdish language as a common language of all Kurd people	0.74		
	One of my great duties is to keep Kurdish language.	0.68		
To be interested in local customs and ceremonies	In my opinion there is no more duty for us as Kurds only to keep our local customs.	0.67	10.1	1.9
	I have loyalty to my local customs.	0.69		
	I am so much interested in recognition of Kurdish customs	0.69		
	It very hard to me if a person (s) insult to Kurdish clothing because it is an indirect insult to me.			
	It very hard to me if a person (s) insult to Kurdish customs because it is an indirect insult to me.	0.69		
	I am proud to see somebody receive different gifts of films with different subject of Kurdish culture at national / international festivals.	0.71		

Table 3: The results of factor analysis of different speeches of ethnic identity

Title of factors	Speeches	Factor load	Specified variant	Special amounts
To be interested in mother land	There is no difference between a Kurd, Lor and /or a Pars. What is important is that all are living in a country.	0.69	9.6	1.74
	It is my proud to be a Kurd.	0.74		
	It is not important for me how other nations and tribes are thinking about Kurds.	0.69		
	I am ashamed to introduce myself as a Kurd.	0.74		
	Any success or fail of other Kurds is not my responsibility.	0.67		
	I am so much interested to know more about my own history.	0.69		
Interested in common living space	If it is possible for me to have an investment in any places throughout the country, I prefer to do it in my homeland.	0.67		
	If it is possible for me to employ any person at equal conditions, I prefer to employ a person with whom I have common language.	0.68		
	I wish to present all my learned things only to those who have common language with me.			
	I prefer to live among Kurds instead of any other places.	0.64	8.14	1.6
	I prefer to live at any place only Kurdistan	0.68		
	I prefer to have Kurd colleagues instead of other persons	0.69		
	It is better to select our friends , neighbors and colleagues from among the own nations.	0.69		
	I a person is living with a person from the same nation, they will face with little problems.	0.70		
KMO=0.87 Bartlett's Test of Sphericity: 1523/67 Sig=0.000				

Research durability

The real meaning of durability is to answer to this question that if a collection of items are measured repeatedly with similar measuring tools or items, is it possible to obtain equal results?
142): In other words, durability means equal results out of repeating the test

In this research and for measuring the internal durability of research tools (questionnaire) Chronbach α is used in this research spss software has been also used for combination among speeches. (Table4&5)

Table 4: The results of factor analysis of different speeches of ethnic identity

Factor	Title of factors	Speeches	Durability in case of omission of speeches	Total durability for each index
Global identity	Common loyalty	-All around the world is my land	0.71	0.71
		-In spite of language, religion and race, all people are respectful.	0.72	
		-I believe in this idea that "All people are like the organs of one body"	0.72	
		-I am responsible against a person only as a person like me.	0.71	
	Common interests	-I am interested in humanistic activities around the world.	0.7	0.71
		-We should try to have a calm and free of war world.	0.71	
		-I respect the charter of human rights.	0.73	
	Social relations preferences	-World values (humanistic) are more important for me than national tribal ones.	0.72	0.72
		-It is better not to confidence in foreigners (Those who are not Iranians)	0.72	
		-I cannot accept to be a friend, colleague or neighbor of a non-Iranian person.	0.74	

Table 5: The results of relevant durability of speeches about global identity

Title of factors	Speeches	Durability in case of omission of speeches	Total durability for each index
To be interested in mother land	I am in love of my land.	0.74	0.75
	Certainly it is a great pleasure for a person to be at service of his/her mother land.	0.75	
	I am ready to work in any place except in my homeland.	0.78	
	I am responsible against my job in my homeland.	0.77	
	In case of providing better job facilities, I may not accept to live in any place rather than my homeland.	0.75	
To be interested in mother language	I am in love of my mother language.	0.74	0.70
	I am proud of Kurdish language as a common language of all Kurd people	0.74	
	One of my great duties is to keep Kurdish language.	0.73	
To be interested in local customs and ceremonies	In my opinion there is no more duty for us as Kurds only to keep our local customs.	0.74	0.74
	I have loyalty to my local customs.	0.79	
	I am so much interested in recognition of Kurdish customs	0.78	
	It very hard to me if a person (s) insult to Kurdish clothing because it is an indirect insult to me.	0.77	
	It very hard to me if a person (s) insult to Kurdish customs because it is an indirect insult to me.	0.70	
	I am proud to see somebody receive different gifts of films with different subject of Kurdish culture at national / international festivals.	0.71	

3. Results

Data analysis: Test of theories

-Globalization is effective on Kurd nation identity

Again we used Pierson meaningful test for testing this theory with regard to different measuring levels of variants. The results indicate that there is a

meaningful and converse relation between globalization and to be absorbed in global identity and identity of Kurd nation. This is because of meaningful level of Sig=0.000 is lower than research α =0.05. Therefore general conclusion is that globalization and interests of respondents in global identity will reduce their interests and love in their own ethnic identity. Type of combination between both mentioned variants is converse. It means that any increase of one item may reduce the other and there is a powerful combination between both variants. r=-0.299. As a result we may confirm research theory on temporary basis which means that there is a meaningful and converse relation between globalization and absorption in global identity and Kurd nation identity. (Table 6)

Table 6

Dependant variant		Ethnic identity
Independent variant		
Globalization & absorption in global identity	Tension	-0.299
	Meaningful	0.000
	Qty	592

Displaying of Regression model

Regression analysis is one of the methods for considering any cause & effect results on other factors. Linear regression is the simplest model as follows:

$Yi=\beta 0 + \beta 1Xi+\varepsilon i$ as dependent variant. By the help of Y as an independent variant and X in this model it is obvious that it is so much effective. Therefore it is possible to use this model in order to find out any effects of Y on X. Then we may study any changes of (X) and globalization and absorbing in global identity (Y) and submission a model between ethnic identity as mentioned in following table.(Table 7)

Table 7:The results of analytical regression statistics of ethnic identity

Combination interval	Specification interval	Modified specified interval	Standard violation	Dourin-Watson test
0.299	0.089	0.088	5.52	1.63

Regarding the statistics mentioned in following table it is possible mention that the obtained unity interval is equal to 0.299. The resulted interval is 0.089 which is a sign of %9 of changes in ethnic identity against globalization and absorption in world identity and also depends upon other factors.(Table 8)

The results of variance analysis show that calculated meaningful level for this statistic was equal to 0.000 and a sign of meaningful level of regression at %99 (Sig=0.000). All diagrams about regression model may confirm the normal condition of remained, as a result it is possible to accept estimated linear regression model. (Table 9)

Table 8: Regression analysis variance of globalization and absorption in global identity and ethnic identity in Kurds

Source of changes	Total of squares	Freedom degree	Average of squares	Statistic of F	Meaningful level
Regression effect	12119.18	1	12119.18	57.855	0.000
Remained	209.747	590	123589.387		
Total	--	591	135708.568		

Table 9: Regression model of globalization effect & Absorption in Global identity on ethnic identity in Kurds

Model		Non-standard interval		Standard Interval Beta	t	Sig
		B	Std. Error			
1	Fixed amount	137.139	4.173	-	32.864	0.000
	Globalization	-0.783	0.103	-0.299	-7.606	0.000
A dependent variant of tribal identity:						

The inserted variant in regression function is the major core of regression analysis as mentioned in table 9. It is possible to calculate regression function by the use of column B and as follows:

Error+(Globalization) 137.139-0.783= Ethnic identity

It means by increasing one unit of globalization and absorption in world identity, there will be a reduction about 0.783 in tribal identity. The relevant t test of regression interval as mentioned in this table show a meaningful interval (Sig=0.000) which is effective in estimation of ethnic identity of respondents.

Second theory: Globalization & Absorption in global identity is effective on Kurd ethnic

In order to test this theory again we used Pierson meaningful test with regard to measuring level of variants. The obtained results show that there is a meaningful and converse relation between globalization and absorption in world identity and dependency feeling in common homeland. This is because of the obtained meaningful level Sig=0.000 is lower than research α =0.05. Therefore the general result is that globalization and absorption of respondents in globalization identity may reduce their interests in their common land.

Type of unity is converse between both mentioned variants. This means that any increase of a variant may lead to another one reduction. Also it is possible to confirm unity of both research theories on temporarily basis which is r=-0.101 as the average

variant. Therefore there is a meaningful and converse relation between globalization identity and dependency & unit feeling to the homeland.(Table 10)

Table10: -Unity test of any relation between globalization & dependency feeling in common land

Dependent variant / Independent variant		Dependency feeling in common land
Globalization & absorption in world identity	Intensity	-0.101
	Meaningful	0.000
	Qty	682

Regression model

In order to consider and submit a model between dependency & unity feeling in common land (Y) we will provide the mentioned model after considering all required indexes of model in following table and investigation of globalization and absorption in world identity (X).(Table11)

Table11:-Analytical regression statistics about dependency &unit feeling in common land

Unit interval	Specification interval	Modified specifying coefficient	Standard violation	Dorbin-Watson test
0.101	0.01	0.009	2.49	1.55

Regarding the mentioned amounts in above-mentioned table, it is possible to say that the unity interval is equal to 0.101. The obtained interval is equal to 0.1 which shows that only %1 of changes in dependency and unit feeling in common land is related to globalization and absorption in world identity. The remained may depend upon other factors.

Third theory: Globalization & absorption in world identity is effective on common language of Kurd nation

In order to test this theory again we used Pierson meaningful test with regard to measuring level of variants. The obtained results show that there is a meaningful and converse relation between globalization and absorption in world identity and dependency feeling in common language. This is because of the obtained meaningful level Sig=0.000 is lower than research α =0.05. Therefore the general result is that globalization and absorption of respondents in globalization identity may reduce their interests in their common language.

Type of unity is direct between both mentioned variants. This means that both may increase and/or decreased at the same time. There is a weak unity between both variants as r =0.095 as the average variant and the research theory is confirmed on temporary basis. Therefore there is a meaningful and direct relation between globalization identity and dependency & unit feeling to the homeland. (Table 12)

Table12:-Unity test of any relation between globalization & dependency feeling in common language

Dependent variant / Independent variant		Dependency feeling in common language
Globalization & absorption in world identity	Intensity	0.095
	Meaningful	0.009
	Qty	749

Regression model

In order to consider and submit a model between dependency & unity feeling in common language (Y) we will provide the mentioned model after considering all required indexes of model in following table and investigation of globalization and absorption in world identity (X).(Table13)

Table13:-Analytical regression statistics about dependency &unit feeling in common language

Unit interval	Specification interval	Modified specifying coefficient	Standard violation	Dorbin-Watson test
0.095	0.009	0.008	2.07	1.59

Regarding the mentioned amounts in above-mentioned table, it is possible to say that the unity interval is equal to 0.095. The obtained interval is equal to 0.009 which shows that only %1 of changes in dependency and unit feeling in common language is related to globalization and absorption in world identity. The remained may depend upon other factors.(Table14)

Table 14:- Regression model of globalization effect on dependency &unit feeling in common language

Model		Non-standard interval		Standard Interval Beta	t	Sig
		B	Std. Error			
1	Fixed amount	11.483	0.546	-	21.02	0.000
	Globalization	0.035	0.013	0.095	2.613	0.009
A dependent variant of tribal identity:						

The inserted variant in regression function is the major core of regression analysis as mentioned in the relevant table. It is possible to calculate

regression function by the use of column B and as follows:
Error +(Globalization) 11.483+(0.035)= Dependency & unity feeling in common language

It means by increasing one unit of globalization and absorption in world identity, there will be a reduction about 0.035 in tribal identity. The relevant **t** test of regression interval as mentioned in this table show a meaningful interval (Sig=0.000) which is effective in estimation of tribal identity of respondents.

Fourth theory: Globalization & absorption in world identity is effective on common customs of Kurd nation

In order to test this theory again we used Pierson meaningful test with regard to measuring level of variants. The obtained results show that there is a meaningful and converse relation between globalization and absorption in world identity and common customs of nation. This is because of the obtained meaningful level Sig=0.000 is lower than research α =0.05. Therefore the general result is that globalization and absorption of respondents in globalization identity may reduce their interests in their common customs.

Type of unity is converse between both mentioned variants. This means that any increase of a variant may lead to another one reduction. There is a powerful unity between both variants as r = -0.172 as the average variant and the research theory is confirmed on temporary basis. Therefore there is a meaningful and converse relation between globalization identity and dependency & unit feeling to the common customs.(Table15)

Table15:-Unity test of any relation between globalization & dependency feeling in common customs

Dependent variant		Dependency feeling in common customs
Independent variant		
Globalization & absorption in world identity	Intensity	-0.172
	Meaningful	0.000
	Qty	749

Regression model

In order to consider and submit a model between dependency & unity feeling in common customs (Y) we will provide the mentioned model after considering all required indexes of model in following table and investigation of globalization and absorption in world identity (X).(Table 16)

Table16:-Analytical regression statistics about dependency &unit feeling in common customs

Unit interval	Specification interval	Modified specifying coefficient	Standard violation	Dorbin-Watson test
0.172	0.03	0.028	4.13	1.88

Regarding the mentioned amounts in above-mentioned table, it is possible to say that the unity interval is equal to 0.729. The obtained interval is equal to 0.03 which shows that only %0.03 of changes in dependency and unit feeling in common customs is related to globalization and absorption in world identity. The remained may depend upon other factors. The modified specification interval in this test is equal to %0.028. Since the statistic amount of Dorbin & Watson is 1.5-2.5, we may result the independency of the remained items.(Table17)

Table 17:- Regression model of globalization effect on dependency &unit feeling in common customs

Source of changes	Total of squares	Freedom degree	Average of squares	Statistic of F	Meaningful level
Regression effect	387.72	1	387.72	22.749	0.000
Remained	12731.228	747	17.043		
Total	13118.948	748	-		

The results of variance analysis show the calculated meaningful level of this statistic is equal to sig=0.000 and a sign of meaningful regression at %99 level.(Table18)

Table18:- Regression model of globalization effect on dependency &unit feeling in common customs

Model		Non-standard interval		Standard Interval Beta	t	Sig
		B	Std. Error			
1	Fixed amount	29.485	1.085	-	27.182	0.000
	Globalization	-0.125	0.026	-0.172	-4.77	0.000
A dependent variant of tribal identity:						

The inserted variant in regression function is the major core of regression analysis as mentioned table 4. It is possible to calculate regression function by the use of column B=29.485 and as follows:
Error +(Globalization) 29.485= Dependency & unity feeling in common customs

It means by increasing one unit of globalization and absorption in world identity, there will be a reduction in common customs. The relevant **t** test of regression interval as mentioned in this table shows a meaningful interval (Sig=0.000) which is effective in estimation of ethnic identity of respondents.

Fifth theory: Globalization & absorption in world identity is effective on common name

In order to test this theory again we used Pierson meaningful test with regard to measuring level of variants. The obtained results show that there is not a meaningful relation between globalization and absorption in world identity and common name. This is because of the obtained meaningful level Sig=0.000 is higher than research α =0.05. Therefore the general result is that globalization and absorption of respondents in globalization identity may reduce their interests in their common name r= -0.041 and it possible to reject research theory on temporary basis. It means that there is not a meaningful unity between globalization and absorption in world identity and dependency feeling in common name.(Table19)

Table19:-Unity test of any relation between globalization & dependency feeling in common name

Dependent variant / Independent variant		Dependency feeling in common name
Globalization & absorption in world identity	Intensity	-0.041
	Meaningful	0.279
	Qty	713

Sixth theory: Globalization & absorption in world identity is effective on common environmental space

In order to test this theory again we used Pierson meaningful test with regard to measuring level of variants. The obtained results show that there is a meaningful and converse relation between globalization and absorption in world identity and common environmental space.

This is because of the obtained meaningful level Sig=0.000 is lower than research α =0.05. Therefore the general result is that globalization and absorption of respondents in globalization identity may reduce their interests in their common environmental space

Type of unity is converse between both mentioned variants. This means that any increase of a variant may lead to another one reduction. There is a powerful unity between both variants as r = -0.355 as the average variant and the research theory is confirmed on temporary basis. Therefore there is a meaningful and converse relation between globalization identity and dependency & unit feeling to the common environmental space.(Table20)

Table20:-Unity test of any relation between globalization & dependency feeling in common environmental space

Dependent variant / Independent variant		Dependency feeling in common environmental space
Globalization & absorption in world identity	Intensity	-0.355
	Meaningful	0.000
	Qty	767

Regression model

In order to consider and submit a model between dependency & unity feeling in common environmental space (Y) we will provide the mentioned model after considering all required indexes of model in following table and investigation of globalization and absorption in world identity (X). (Table 21)

Table21:-Analytical regression statistics about dependency &unit feeling in common environmental space

Unit interval	Specification interval	Modified specifying coefficient	Standard violation	Dorbin-Watson test
0.355	0.126	0.125	5.89	1.89

Regarding the mentioned amounts in above-mentioned table, it is possible to say that the unity interval is equal to 0.355. The obtained interval is equal to 0.126 which shows that only %0.126 of changes in dependency and unit feeling in common environmental space is related to globalization and absorption in world identity. The remained may depend upon other factors. (Table22)

Table 22: Regression model of globalization effect on dependency &unit feeling in common environmental space

Model		Non-standard interval		Standard Interval	t	Sig
		B	Std. Error	Beta		
1	Fixed amount	43.344	1.539	-	28.159	0.000
	Globalization	-0.39	0.037	-0.355	-10.505	0.000
A dependent variant of tribal identity:						

The inserted variant in regression function is the major core of regression analysis as mentioned table 4. It is possible to calculate regression function by the use of column B=29.485 and as follows:
Error + (Globalization) 43.344 - 0.39 = Dependency & unity feeling in common environmental space

It means by increasing one unit of globalization and absorption in world identity, there will be a reduction in common environmental space. The relevant **t** test of regression interval as mentioned in this table shows a meaningful interval (Sig=0.000) which is effective in estimation of common environmental space of respondents.

4. Discussions

General result is the increase of dependency and interest of respondents in their common language by globalization and absorption. Also according to the obtained results it is revealed that there is a

meaningful and converse relation between globalization and world absorption and independent variants (dependency & unit feeling in common homeland, common customs and common environmental space. Therefore it is concluded that globalization and absorption may reduce the rate of ethnic identity variants in respondents. As a result and upon the obtained results of this research, this viewpoint is completely related to the obtained results out of statistic society of research. This is a sign of application this idea in statistic population. Upon the mentioned results if Kurdish nation maintain their globalization process they should bear different parameters including equal alphabet & script, world potentials of culture & language, lack of exclusivity to a special country, presence of a rich written heritage and powerful political support in order to maintain its position in world social relations network with an integrated context. In other words ethnic identity of Kurd nation may help them in revival of their ethnic identity due to the lack of exclusivity to a special country and by a rich written heritage in Iraq Kurdistan and with lower degree in Iran Kurdistan. Also the presence of different T.V. networks in local language broadcasting in satellite may assist such a resistance.

There are a lot of efforts in the field of renovation and collection and archiving of folkloric sings and T.V. shows at different areas of Kurdistan are some other efforts for this purpose. Furthermore any presence of Kurdish language institutes and also teaching 2 units of local language at universities and also Kurdish Language University at Kurdish areas of Iraq and changing it into second official language of country are the other aspects for assisting the revival of Kurdish language.

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7/17/2011

Gastroduodenal Masses: Multidetector Computed Tomography (MDCT) Findings And Differential Diagnosis With Pathologic Correlation

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Abstract: To study the role of multidetector CT for the evaluation of gastroduodenal masses and differential diagnosis. All patients in the present study (25 patients) were examined with dual slice CT GE using diluted gastrografin oral intake and IV contrast injection of non-ionic contrast agent. The study was included 25 patients suffered from gastroduodenal masses undergoing abdominal US, endoscopic examination, multidetector computed tomography (MDCT) as well as biopsy and histopathological examinations. 20 patients were diagnosed as gastric lesions and 5 patients were diagnosed as duodenal lesions. The patients examined were 17 males and 8 females with the age ranging from 32 to 79 years and the mean age 55 years ± 2SD. Conclusion: Multidetector Computed Tomography (MDCT) has a great value for evaluation and differential diagnosis of gastro duodenal masses with well correlation with histopathological findings.
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Key words: MDCT • pathology • correlation

1. Introduction:

Recent three-dimensional multidetector row computed tomography (3D MDCT) can provide detailed images of gastro intestinal tumors, including its general contour, location and depth [1].

MDCT may be a reliable means of noninvasive diagnosis in the care of patients with endoscopically detected giant gastric folds and may be useful for differentiating benign from malignant disease[2].

Recent advances in multidetector computed tomography (CT) with multiplanar reformation (MPR) provide a powerful tool for identifying gastric wall invasion and the perigastric extent of gastric cancer. In addition, MPR images confer advantages in the assessment of both intra- and extraluminal processes of the gastric wall and the evaluation of more distant regions, such as the paraaortic lymph nodes and other abdominal organs [3].

The assessment of direct transmural and extraserosal spread of disease (T staging) and nodal involvement (N staging) has improved markedly with the advent of 3D MDCT with its excellent spatial and temporal resolution and its ability for multiplanar image reconstruction[4].

The aim of this work to study the role of multidetector CT in the evaluation of gastroduodenal masses and differential diagnosis with histopathological findings correlation.

2. Patients and Methods

Patients:

The study included 25 patients with the age ranging from 32 – 79 years and the mean age 55 years ± 2SD. 17 patients (68%) were males and 8 patients (32%) were females.

All the patients were complained from different gastrointestinal manifestations diagnosed by endoscopy (25 patients) in addition to abdominal US (25 patients) with biopsy and histopathological examinations.

CT imaging technique:

The study was performed on CT scan Hispeed Dual slice GE, USA.

The patients were given 750 ml of diluted gastrografin 20 minutes before scanning for gastric duodenal distension and opacification.

In addition to the oral contrast agent (750 ml gastrografin), 100 ml of non-ionic contrast material (Optray 350) were injected intravenously at rate of 3ml/second.

3. Results

Twenty patients were diagnosed as gastric lesions and five patients were diagnosed as duodenal lesions. Gastric carcinoma patients were 12 patients divided into two categories:

Early adenocarcinoma: no local invasion or metastases.

Advanced adenocarcinoma: with local invasion or metastases.

MDCT detection rate for early adenocarcinoma was 40% (2/5) and 100% (7/7) for advanced adenocarcinoma with the all detection rate

was 75 % with statistically significant difference (P less than 0.05) for early and advanced adenocarcinoma detection rate.

Gastric adenocarcinoma findings were mass lesions appeared as focal defects at contrast filled stomach (Figs. 1 , 2).

Fig.(1): 41 years old women with gastric mass lesion proved histopathologically as gastric adenocarcinoma.

Fig.(2): 50 years old man with advanced gastric mass lesion proved as gastric adenocarcinoma by biopsy.

Gastric lymphoma: detection rate by MDCT was 75% (3/4) and one patient from the four patients demonstrated no abnormality at MDCT, the abnormality in the three patients were diffuse or segmental gastric thickening , however, from the three patients only two patients demonstrated lymphadenopathy (50%) (fig. 3 &4) and one patient demonstrated ulcer (25%).

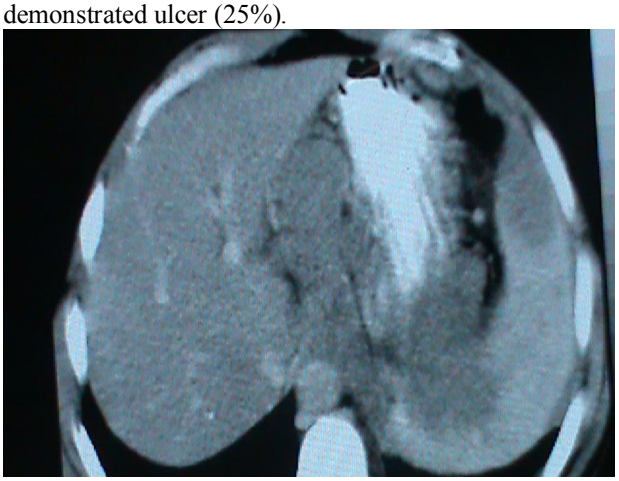


Fig. (3 A): 63 years old man with gastric diffuse mural thickening proved as gastric lymphoma.

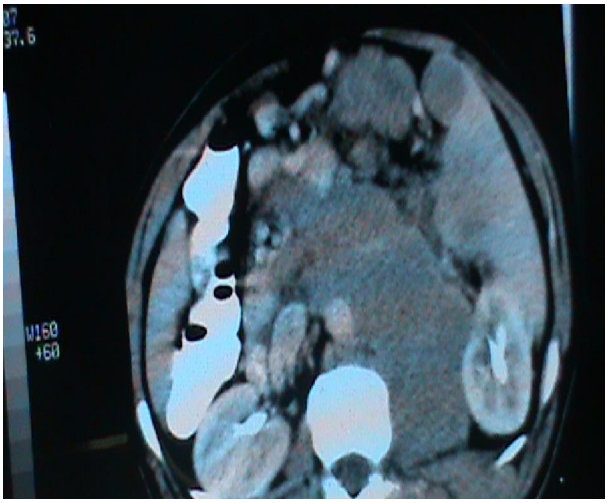


Fig.(3 B):The same previous patient with paraaortic extensive lymphadenopathy.

Fig. (4A):

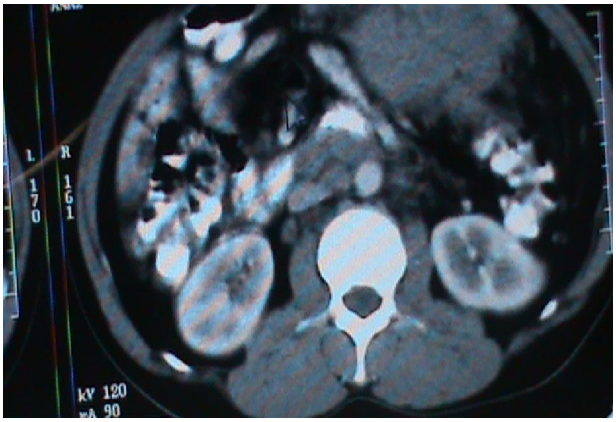


Fig.(4B)

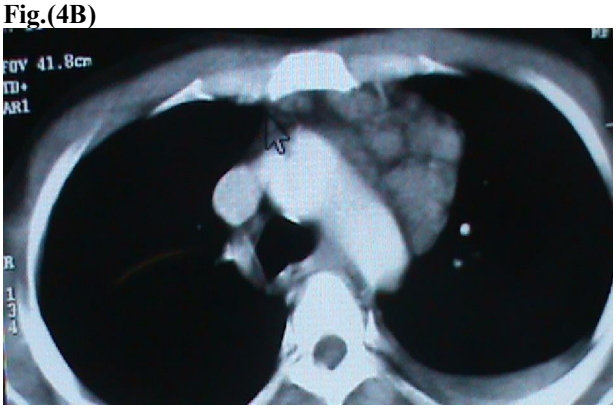
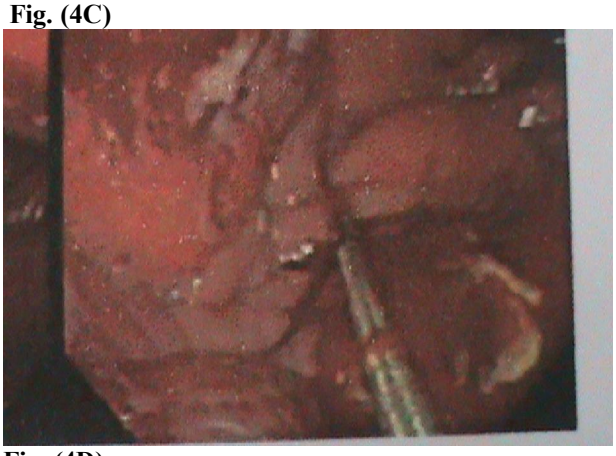


Fig. (4C)



Fig, (4D)

Fig.(4): 36 years old man with extensive gastric mural thickening (A), paraaortic lymphadenopathy (B), mediastinal lymphadenopathy (C) and endoscopy findings (D) proved as gastric lymphoma by biopsy.

Gastro intestinal stromal tumors (GIST): the abnormality noted in the 3 patients (detection rate 100%) with one small size appeared as mass with homogenous attenuation and other two patients appeared as large masses one of them showed heterogeneous pattern and cystic changes due to necrosis and hemorrhage (Fig. 5).

Masses of GIST appeared project exophytically and/or intraluminally with no evidence of mucosal ulceration at our study.

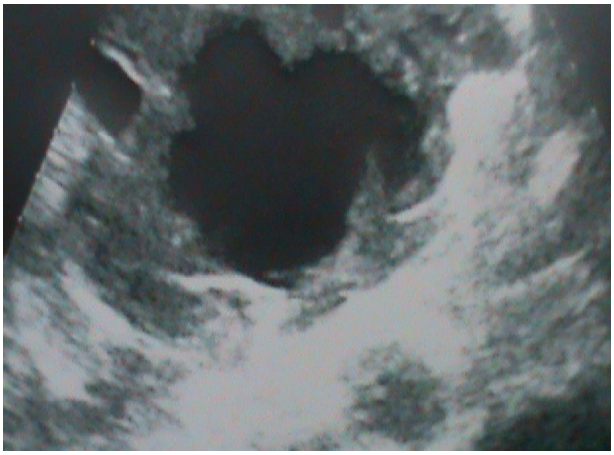


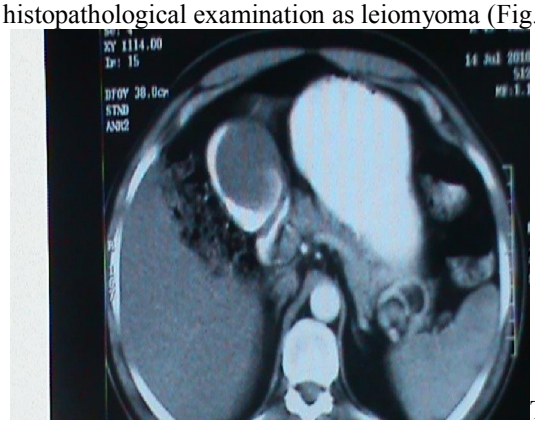
Fig. (5A)

Fig. (5B)

Fig. (5C)

Fig. 5: 64 years old women with mural gastric mass projected exophytically and intraluminally with cystic component at US (A), MDCT (B) and homogenous enhancing lesion at (C) proved histopathologically as GIST.

Gastric leiomyoma: only one patient presented with well-defined homogenous mass proved by histopathological examination as leiomyoma (Fig. 6).



The 5

Fig. (6) 41 years old man with gastric well-defined homogenous submucosal mass proved histopathologically as leiomyoma.

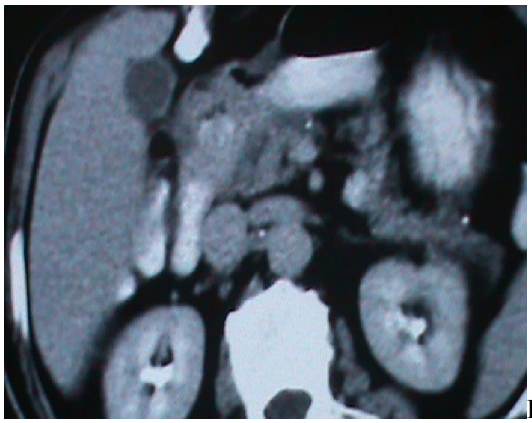


Fig. (7A)

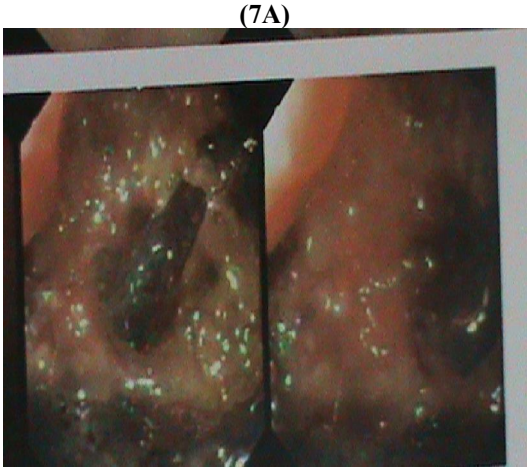


Fig. (7B)

Fig. (7): 76 years old man with duodenal mass at MDCT (A) and endoscopy (B) proved histopathologically as duodenal adenocarcinoma.

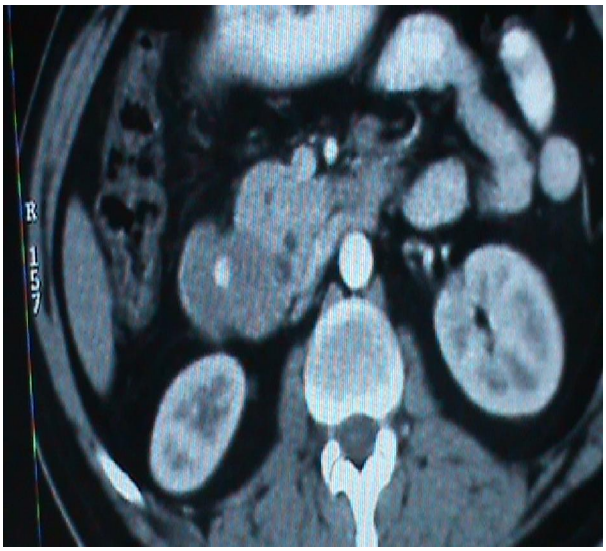


Fig. (8A)

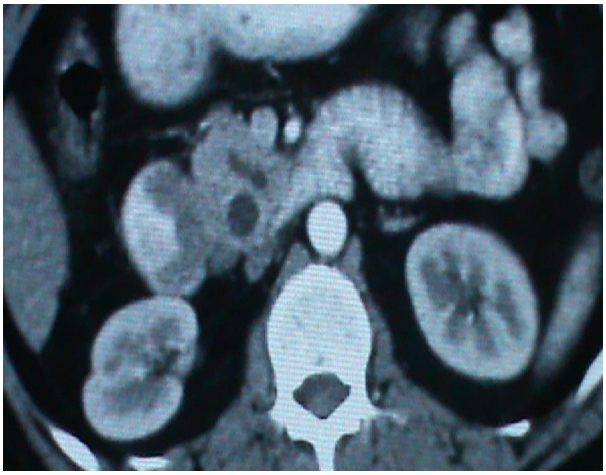


Fig. (8B)

Fig. (8):50 years old man with duodenal mass extending to pancreatic head proved as duodenal carcinoma by biopsy.

Patients with duodenal masses were detected by MDCT and diagnosed as suspicious of carcinomas (Fig. 7) with one patient there is extension of the mass to pancreatic head (Fig. 8) and proved histopathologically as duodenal carcinomas aside from one carcinoid (Fig. 9).

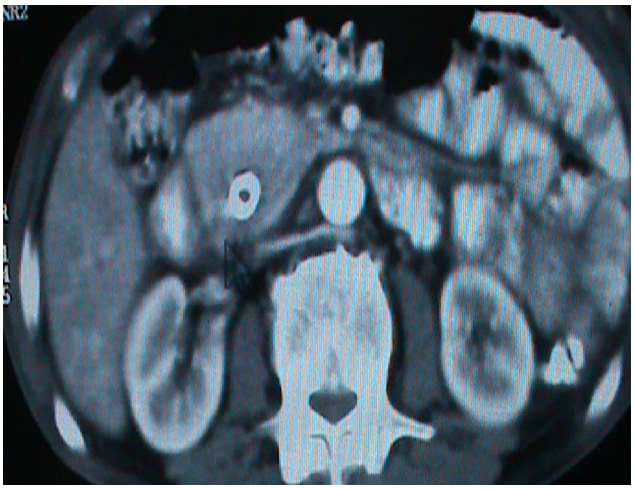


Fig. (9A)

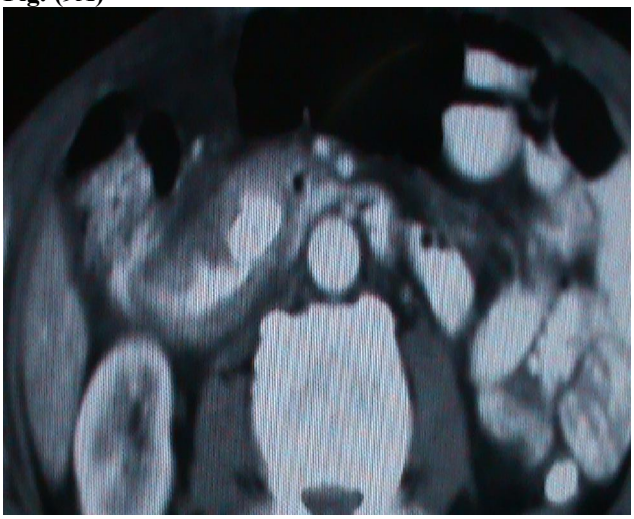
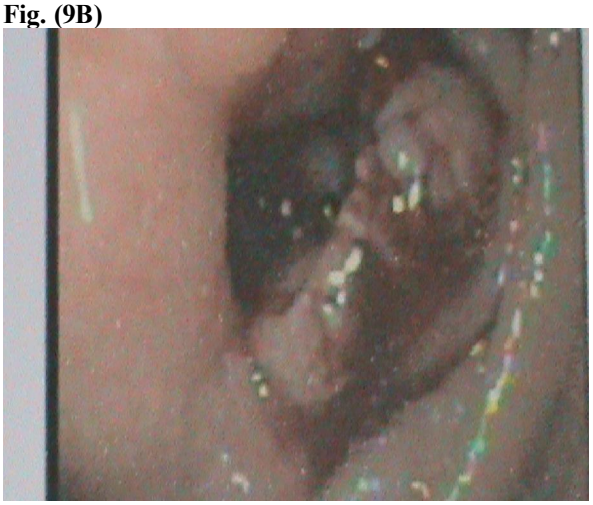


Fig. (9B)



F **Fig. (9C)**

Fig. (9): 65 years old man with MDCT showed duodenal mass (A) exhibits contrast filling defect (B) as well as endoscopic findings (C) proved by biopsy as duodenal carcinoid.

Based on histopathological examinations the results were:

- 12 cases gastric adenocarcinoma.
- 4 cases gastric lymphoma.
- 3 cases gastro intestinal stromal tumors (GIST).
- One case gastric leiomyoma.
- 4 cases duodenal carcinoma.
- One case duodenal carcinoid.

4. Discussion

(Dicken and others 2005)[6] mentioned ninety percent of all tumors of the stomach are malignant, and gastric adenocarcinoma comprises 95% of the total number of malignancies .

In our study adenocarcinoma represented about 60% from total number of gastric masses (12/20) likely related to the relative small number of cases in this study.

Detection rates for adenocarcinoma in this study were 40% for early adenocarcinoma which considered early if there is no evidence of local invasion or metastases and 100% for advanced adenocarcinoma where there is local invasion or metastases, these findings are relatively comparable to previous study by (Kensaku *and others 2005*) [7] which reported 41% and 96 % for detection rate of early and advanced gastric adenocarcinoma, respectively by MDCT.

As regarding to gastric lymphoma detection rate was 75% as diffuse or segmental thickening with 40% showed lymphadenopathy and 25% showed ulcer, these findings are relatively correlated with previous study (Dongil *and others 2002)* [8] which reported 69% detection rate, lymphadenopathy 40% and ulcer 22% in study included 58 patients with gastric lymphomas.

Gastrointestinal stromal tumors (GIST) showed well demarcated masses project exophytically and/or intraluminally as expressed in (Miettinen and Lasota study 2001) (9), these findings were noted in all patients (100%) detection rate in our study, on the other hand, (Pidhorecky and others 2000) (10) reported detection rate 87%.

(Coulier *and others 2007)*[11] reported the primary carcinoid tumor clearly appeared as a contrast-enhancing intraluminal lesion in all cases except in one case in which the primary lesion remained unlocalized and in another in which the primary tumor finally appeared infracted at gross anatomy. The maximal tumoral enhancement was obtained in 3 patients imaged during the acute arterial phase. 6/7 patients had multiple prominent mesenteric nodal metastases, all also appearing as hypervascularised enhancing masses. In 4/7 patients the nodal metastases represented the major finding being much prominent and larger than the primary

tumour. Signs of retractile mesenteritis with soft tissue stranding, retraction and stellate pattern of the mesentery were found around the mesenteric metastases in 5/7 patients and direct incarceration of vessels were found in 3 cases, these findings can't correlated with carcinoid findings in our study for the very small number of carcinoid patients in this study (one patient).

Conclusion

Recent advances in CT technology and 3D imaging software have sparked renewed interest in using CT to evaluate gastroduodenal masses. Detailed CT examinations of the stomach and duodenum can routinely be performed with oral contrast intake, along with a rapid intravenous contrast material bolus and the thin collimation that is possible with new multidetector row CT scanners. MDCT is a valuable tool for the evaluation and differential diagnosis of gastro duodenal masses with high correlation with histopathological findings aside from early gastric carcinoma which showed relative low detection rate that open the way for more studies and research in the future to try find a way to solve this problem.

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7/17/2011

Application of Health Belief Model in Prevention of Breast Cancer among female staffs of Islamic Azad University-Tonekabon branch in Iran

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Abstract: Background and Objectives: Nowadays, malignancy is considered as one of the most important health burden in the world. In this study, with respect to the importance of training in order to promote preventive behaviors, the application of a training program based on health belief model was surveyed. A semi–experimental study was conducted on 100 female staffs of Islamic Azad university-Tonekabon branch, Mazandaran, Iran. Samples were randomly collected and classified as intervention (case) and control groups by interviewing using a health belief model–based questionnaire. Both groups completed the questionnaire twice within a three months period of time before and after training of intervention group. Data was analyzed by statistical tests and SPSS software (versions 11.5 and16). Based on results obtained by this study, two groups had no significant difference in terms of demographic variables. However, a significant difference was observed among scores of different constructs of this model such as perceived severity, benefits, and barriers before intervention, and scores of knowledge, attitude, perceived benefits and barriers, and act guidance after intervention ($p<0.05$).The findings of this study imply that the present HBM based intervention has had a positive effect on increasing women's knowledge, attitude, and behaviors regarding breast cancer.
[Afsaneh Gity [1], Davood Shojaeizade [2], Abas Rahimi Froshani. **Application of Health Belief Model in Prevention of Breast Cancer among female staffs of Islamic Azad University-Tonekabon branch in Iran.** Journal of American Science 2011;7(8):633-640]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Health Belief Model, Prevention, Breast cancer, Attitude.

1. Introduction

Malignancy is currently considered as one of the most important health problems in the world (Ebrahimi,2007). Cancer includes more than 200 different diseases, all involved in malignancy (NCDC,2004). Breast cancer has become the third cause of mortality and the second group of non-infectious chronic diseases among the women all around the world, so that every one out of eight women will develop this disease during her lifetime. However, this cancer is possible to be prevented or treated in many cases (Ghaem Maghami F , 1998). The prevalence of this cancer in Iran is estimated to be 17.1%, which comprises 33% of all women's cancers and 19% of their cancer mortality. Around 57% of Iranian patients are diagnosed at second stage of cancer, 25% at third stage and only 18% at first stage. However, in developed countries, patients are mostly diagnosed at the first and second stages (http://pezeshk.breast cancer .com). It is estimated that 90% of breast masses are detected by women's self-examination, of which 20-25% are malignant (Sadegh Nejad F 2001 5). The prevalence of breast cancer has been increasing during the past two decades. Researchers in the USA Cancer Institute have addressed it to the changes in impregnating models such as late pregnancy and having fewer children (Ghaem Maghami F,1998 3). Fear, anxiety, shame, and lack of training are considered as the important factors that restrain women to do breast self–examination (Nustas W ,2002 ; Bostick RM , Sprafka JM ,Virjin BM ,1993). A study done on 600 female staffs of schools located in the city of Tehran showed that breast self–examination training has a significantly positive effect in early detection of masses and increasing health beliefs in all levels ($p<0.001$) (Tahvildari S ,2005).Since breast cancer threatens women's health, a research priority has been given to it by the health authorities in different countries including Iran. However, due to the cultural environments in countries like Iran, it is not possible to use media to train women regarding early diagnosis of this disease. Therefore, it is necessary for authorities to introduce broad training programs for secondary prevention. The importance of secondary prevention is highlighted as the early prevention opportunities are limited. On the other hand, regarding the need of public to be trained, training of breast cancer can play an important role in prevention Nevertheless, due to lack of knowledge regarding all different factors that influence breast cancer, introducing different aspects of disease to the

public with emphasizing on the training for self-examination would plays an important role in prevention and on time diagnosis(Porzand A , 1998). Hence, surveying the level of public knowledge, their beliefs, and behaviors in any population would have an important role in designing programs for troubleshooting of health training in society (Ebrahimi , 2007). On the other hand, screening methods can be used as alternative to accomplish early prevention and decrease mortality. Nevertheless, despite attempts, 20-60% of women do not concur with screening methods (Ghaem Maghami F, 1998). Also, in spite of enlightening of women regarding the importance of early diagnosis of breast mass or any unusual symptoms and encouraging them to do self–evaluation examinations, yet more than 20% of women refer to doctor with an advanced level of breast cancer (Love S , 1998). The first stage in health training is to enhance public knowledge regarding prevention methods to improve their health (Shojaee Ji Tehrani H , 1999). Health Belief Model (HBM) is widely used in the framework of anticipated studies of health–related behaviors (shojaaezadeh D , 2000). It is a broad method that concentrates more on prevention than control, even though in secondary prevention, it more focuses on control of disease. Therefore, according to this model, a person goes through a health action only when he/she understands the threat, susceptibility, severity of disease, and health value of that action). (shojaaezadeh D ,2000). This study aimed to investigate the effect of the HBM on enhancing knowledge and improving attitude and performance of women against breast cancer. Similar research done by Oyewopo et.al (211), Jourkeshet.al (2011), Ashour et.al (2011) and Abd El-Aziz et.al (2011).

2. Material and Methods

A semi–experimental case–control study was conducted on 100 female staffs of Islamic Azad University-Tonekabon branch located in Mazandaran province, Iran. They were classified as intervention (case) and control groups each including 50 individuals. A questionnaire was used as data collection tool. The questionnaire was compared with the authentic references and its validity was confirmed by the health science experts at university of Tehran. To complete the questionnaire, all participants were interviewed based on health belief model. The interview took place twice within a three months period of time before and after training of intervention group. The questionnaire included three sections. The first section was related to the demographic data (table 1); the second to measure the knowledge of participants regarding breast cancer and its prevention by screening methods; and third to determine their attitude toward breast cancer based on the health belief model (table 3). After completing the first questionnaire by all participants, the intervention group was divided to four subgroups each including 7-15 individuals and three collective discussions were conducted among them with training purposes under supervision of the researchers. Each session lasted three hours and the auxiliary training tools included film, moulage, and pamphlet. All participants were interviewed again to fill up the questionnaire three months after the first interview. The parameters were classified in two clusters and compared by paired–*t* test, while others were classified in three clusters and compared by one-side variance analysis. All results were analyzed by SPSS software (versions 11.5 and 16) (table 1).

3. Results

The demographic data obtained by this study is shown in table 1. The knowledge and HBM model were first compared by independent test before and after training in both case and control group, which showed significant association ($p<0.01$) (table 3). The comparison of all scores before training showed no significant association between case and control groups except those for perceived benefits, perceived barriers and severity ($p<0.05$) (table 4). However, all scores except perceived susceptibility and severity showed significant association between control and test groups after training ($p<0.05$) (table 5). For surveying the relationship between demographic factors with level of knowledge and attitude of subjects toward breast cancer, two clustered and three clustered parameters were measured by pair–T test and variance analysis, respectively. These parameters ors were specified as the most important factors influencing breast cancer ($p<0.001$) (table 2).

4. Discussions

This study evaluated the level of knowledge and attitude of female staffs of a university toward breast cancer based on Health Beliefs Model (HBM). Some parameters including age, marital status, education status, house owning, level of income, being believe in milking, records of breast cancer among family and relatives, and being affected of the participant were used to compare case and control groups before and after training (table 1). Improvement in level of knowledge and attitude toward breast cancer in the framework of HBM has a positive effect on preventive behaviors in women. The findings of our study confirmed that the HBM–based program positively affects the susceptibility, severity, and perceived benefits and barriers, and act guidance. This study showed that the mean scores of

knowledge and preventive methods were significantly higher in cases than controls both before and after training ($p<0.001$) (table 3). These results are consistent with those obtained by some other researchers. The research performed by Rahmati showed that the mean scores of knowledge and preventive methods achieved by cases were higher than average ($p<0.001$) (Rahmati Najar Kaliaee F, 2002). The study done by Hee–Soon Ivon (2006) on Korean women showed that intervention group had an increased level of knowledge about breast cancer after training in compare to control group (Hee- Soon Ivon PHD A , 2006). The higher level of knowledge is in direct relation with early diagnosis and increasing the chance of treatment being more effective. Since having knowledge about a topic is a prerequisite to decide for the correct attitude and suitable behavior regarding that topic, having knowledge about breast cancer can help in early diagnosis of this disease at first stages and increase the life duration of infected women (Harris R , Leininger L , 1995). The finding of this study confirms that the HBM–based program positively affects the susceptibility, severity, perceived benefits and barriers, and act guidance of case group. Studies done by some other researchers have shown that women avoid self-examination due to some reasons including disbelieving, forgetting, fear, and husband's disagreement (Bostick RM ,Sprafka JM, Virning BA ,Potter JD ,1993 ;Manfredi C, Warnecke RB, Graham S ,Rosenthal S,1997;Thomas EC, 2004).Based on the study done by Jahangardies, 60.5% of subjects had primary and intermediate education, and their level of knowledge about breast cancer was affected by their level of education (Jahangiri L ,2007). The mean scores of two groups measured in their study also showed significant relationship between perceived benefits and preventive methods before and after training ($p<0.001$) (table3). In study done by Jahangardies50.5%of subjects a chieved higher mean scores for perceived benefits after training ((Jahangiri L ,2007). In the study done by Georrgiar Salder regarding perceived benefits as a determinant factor in applying preventive methods, 94% of women agreed with the early detection of breast cancer (Salder GR , Dhanjel SK , Shah NB , KO C Anghel M , 2001).In our study, the mean scores of susceptibility toward breast cancer and preventive methods showed significant association in both case and control groups before and after training ($p<0.001$) (table 3). Our results also showed that subjects had a higher susceptibility toward expected matter, while concerning factors of cancer were different and unclear in some cases (Breast Cancer International Research Group, Jornal, 2007). The study done by Rahmati (2002) showed that case group achieved a high score in perceived susceptibility toward breast cancer (Rahmati Najar Kaliaee , 2002). This result emphasizes on the need of increasing knowledge to better perform preventive methods in breast cancer.The susceptibility of intervention group was effective in adopting preventive behaviors, and successful prevention depended on data of personal susceptibility and its risk in every individual .In addition, perceiving severity of disease and its symptoms and results were of the main components of the health belief model (HBM), which were effective in adopting preventive behaviors.The surveying of mean scores of perceived barriers showed a significant relationship between breast cancer and its methods in both case and control groups before and after training($p<0.001$) (table 3). The factors preventing self–examination included fear, anxiety, shame and lack of skill about how to do it correctly (Nustas W ,2002 ; Bostick RM , Sprafka JM ,Virjin BM ,1993). It is important to notice that perceived barriers are considered as the key component of health belief model, as it can be used to predict the person's performance. It is also considered as an important factor in acquiring preventive behaviors and in reducing barriers by increasing knowledge. The authentic and successful prevention depends on the information of barriers (Theory At a Glance ,Seconde Edition). The findings also showed a significant difference between mean scores of perceived severity before and after training in intervention group ($p<0.001$) (table 3). In study done by Jahangardi, the mean score of problem severity increased after training. In this study, a statistically significant increase in mean score of problem severity was also observed in intervention group ($p<0.001$), while no significance was seen among control group (Jahangiri L ,2007). In her study, the mean scores of act guidance increased after training (Jahangiri L ,2007). The findings of our study also showed a significant relationship between mean scores of act guidance in female staffs before and after training in both intervention and control groups ($p<0.001$) (table3). Since this parameter considers health staff as data source, it seems that these staff can play an important role in education of society regarding the prevention of cancer. Health personnel can have an effective influence on people who refer to them. These results are consistent with those studies which have classified health personnel and media as the first and second priority in education of society, respectively (Jahangiri L ,2007). However, Dundar and Ozmen showed that, as a source of education, media including Radio and Television are prior to the health personnel and doctors ($p<0.001$) (Dundar, Ozmen,2006). Degree of

correlation between conceived benefits with the women who had or had not record of breast cancer among their family in case group was significant both before and after training, while in control group it was significant only before intervention (p<0.001) (table2).

Table 1. Demographic data of cases and control groups of studied female staffs of Islamic Azad University-Tonekabon branch .

kind	groups	Case	Percentage	Control	Percentage more	P- valu
Age(30-20) (40-31)(>40)	(3groups)	agemoreof41	58%	31-40	50 %	p<0/001
underdiplom Diploma University	(3 groups)	UniversityEducation	78%	Education university	90%	P<0/001
Dead hausband Marriage Not marriage	(4groups)	Marriage	78%	Marriage	88%	P<0/001
Without hausband having Hause not having Hause	(2groups)	Hausing	90%	Hausig	82%	P<0/001
Believeinmilking(not) (Mide)	(3groups)	(verymore)	74%	Believein milking(very more)	78%	P<0/001
Have Very more Having Or not	(2groups)	Breastcancer in persons	94%	Breast cancer in persons	92%	P<0/001
(not) near their relative very their relative not near	(3groups)	breastcancer in their relative	78%	breast cancer intheir relative	74%	P<0/001
(300 -500) 700-500 >700	(3groups)	income	46%	income	66%	P<0/001

Table 2. Comparison between mean scores of different parameters based on their significance among trained and untrained female staffs of Islamic Azad University-Tonekabon branch .

Dependent variable	variable	P-value
Perceived benefits after intervention (case)	Having breast cancer or not	P<0/05
perceived Severity after intervention (case)	education	P<0/05
Perceived benefits after intervention (control)	education	P<0/05
Perceived Severity before intervention (control)	marriage	P<0/05
Knowelge after intervention (case)	breast cancer in their relative	P<0/05
Perceived benefits after intervention(case)	breast cancer in their relative	P<0/05
Attitude intervention befor and after(control)	breast cancer in their relative	P<0/05
Perceived barrier intervention after(control)	breast cancer in their relative	P<0/05
Act guidance before(control)	income	P<0/05

Table 3. Comparison between mean and standard deviation of different parameters before and after applying of HBM among trained and untrained female staffs of Islamic Azad University-Tonekabon branch.

	knowelge		Attitude		Perceived benefits		barrier Perceived		Perceived susceptibility		perceived Severity		Act guidance	
Before	case	witness	case	witness	case	witness	case	witness	case	witness	case	witness	case	witness
Mean	8.640	8.800	33.800	33.560	9.880	10.560	4.900	4.420	5.080	4.440	8.740	9.920	9.300	9.280
Standard Deviation	2.505	3.270	4.393	4.936	1.825	1.296	1.359	1.667	1.523	1.214	2.553	2.146	2.832	2.618
After														
Mean	19.260	8.820	47.300	42.380	14.460	11.060	5.600	5.080	6.340	4.980	10.300	10.482	15.680	18.100
Standard Deviation	2.716	3.293	3.643	4.869	1.853	1.095	1.690	1.275	1.205	1.151	1.717	1.593	1.973	2.476
Meaningful Comparision Before And after	P<0.001		P<0.001		P<0.001		P<0.001		P<0.001		P<0.001		P<0.001	

Table 4. Comparison between all scores before applying HBM model among trained and untrained female staffs of Islamic Azad University-Tonekabon branch.

Before	grops	N	mean	sdt	F	t	df	Sig. (2- tailed)
Knowelge	Case	50	338000	4,39387		.257	98	.798
	Control	50	335600	4,93699	.187		69698	
Attitude	case	50	86400	2,50518	2.121	-.275	98	.784
	Control	50	88000	3,27015			91.779	
Perceived benefits	case	50	98800	1,82544	6.706	- 2.148	98	.034
	Control	50	105600	1,29615			88.395	
Perceived barrier	case	50	50800	1,52315	1.799	2.323	98	.022
	Control	50	44400	1,21487			93.383	
Perceived susceptibility	case	50	49000	1,35902	1.703	1.578	98	.118
	Control	50	44200	1,66709			94.176	
perceived Severity	case	50	87400	2,55399	1.715	-2.501	98	.014
	control	50	99200	2,14609			95.175	
Act guidance	case	50	99200	2,82301	. 160	-.037	97.453	.971
	Control	50	99200	2,61893			98	

Table 5. Comparison between all scores after applying HBM model among trained and untrained female staffs of Islamic Azad University-Tonekabon branch

after	grops	N	mean	sdt	F	t	df	Sig. (2- tailed)
Knowelge	Case	50	47.3000	364356486927	1.251	5.721	98	.001
	Control	50	42.3800				90.775	
Attitude	case	50	19.2900	2,716623.29310	3.139	17.293	98	.001
	Control	50	8.8200				945582	
Perceived	case	50	14.4600	185395	17.731	11.164	98	.001
benefits	Control	50	11.0600	1.09563			79505	
Perceived	case	50	6.3400	1.20560	.063	5.768	98	.001
barrier	Control	50	4.9800	1.15157			97.795	
Perceived	case	50	5.6000	1.69031	8693	1.737	98	.086
susceptibility	Control	50	5.0800	1.27520			91.129	
perceived	case	50	10.3000	1.17172	.156	-.543	98	.588
Severity	control	50	10.4800	1.59387			97.460	
Act	case	50	15.6800	1.97370	1.416	-5.404	98	.001
guidance	Control	50	18.1000	2.47642			93.354	

Table 6. Descriptive statistics of mean scores and standard deviations of HBM model among case and control women studied in Islamic Azad University-Tonekabon branch in the year 2010.

case					control				sig
Before and after intervention	upper	Lower	Std.	Mean	Upper	Lower	Std.	mean	
knowelge before	9.3520	7.9280	2.50518	33.8000	9.7294	7.8706	4.93699	8.8000	.78
knowlege after	20.0321	18.4879	2.71662	47.3000	9.7559	7.8841	4.86927	8.8200	.001
Attitude before	35.478	32.5513	4.39387	8.6400	34.9631	32.1569	3.27015	33.5600	/798
Attitude after	48.3355	46.2645	3.64356	19.2600	43.7638	40.9962	3.29310	42.3800	/001
Perceived benefits before	10.3988	9.36121	1.82544	9.8800	10.9284	10.1916	1.29615	10.8600	034/
Perceived benefits after	14.9869	13.9331	1.85395	14.4600	11.3714	10.7486	1.09563	11.0600	000/
Conceived barriers before	5.5129	4.4671	1.52315	5.0800	4.7853	4.0947	1.21487	4.4400	022/
Conceived barriers after	6.6826	5.9974	1.20560	6.3400	5.3073	4.6527	1.15157	4.9800	/001
Conceived sensitivity before	5.2862	4.5138	1. 35902	4.9000	4.8938	3.9462	1.66709	4.4200	/118
Conceived sensitivity before	6.0804	5.1196	1.69031	5.6000	5.4424	4.7176	1.27520	5.0800	/086
Problem intensity before	9.4658	8.0142	2.55399	8.7400	10.5299	9.3101	2.14609	9.9200	/014
Soverity after intervention	10.7880	9.8120	1.71726	10.3000	10.9330	10.0270	1.59387	10.4800	/588
Act guidance before	10.1023	8.4977	2.82301	9.3000	10.0243	8.5357	2.61893	9.2800	/971
Act guidance after	16.2409	15.1191	1.97370	15.6800	18.8038	17.3962	2.47642	18.1000	/001

According to the study done by Rahmati (2002), 29.3% and 11.1% of subjects had positive record of disease among their family members and relatives (Rahmati ,2002), respectively; while in study done by Banayan ,1.5% of subjects pointed to have breast cancer patients among both their family members (either sister or mother) and relatives(Banayan ,2005). The relationship between

scores of severity and level of education showed significant association in case group after training ($p<0.001$) (table2), while significant correlation was observed between scores of perceived benefits after training in control group ($p<0.001$).

In the study done by Parsaee (2001) regarding the effect of education on prevention of breast cancer, 47.2% of participants were uneducated and showed that the level of education has a significant effect on prevention of breast cancer. This study showed a meaningful relationship between knowledge and the importance of training in creating severity ($p<0.001$) (Parsaee ,2001). Our study also showed that the level of education has high effect on perceived severity ($p<0.001$) (table 2). This study also showed that perceived severity is affected by level of education which is consistent with the study done by Jahangardi ($p<0.001$)(Jahangiri L ,2007). Also, a significant correlation between scores of perceived severity and marital status was observed before training in control group ($p<0.001$) (table 2). In a study done by (Hubbel FA ,Mishra SI ,Chvez LR ,Valdez RB ,1995) mammography was applied on 430 women and marriage was considered as an effective factor. However, this factor showed no significant association in our study(Hubbel FA ,Mishra SI ,Chvez LR ,Valdez RB ,1995). Correlation between perceived severity score was significant in groups who were believe in milking before training in control group, while this factor showed no significant association in other group. The scores of knowledge and perceived benefits showed significant relationship after training in case group with having record of breast cancer in relatives ($p<0.001$) (table 2); while the score of attitude showed a significant association before and after training in control group with record of breast cancer in relatives. In this study, 78% of case group and 74% of control group did not point to have any record of breast cancer in their family, while 20% of case group had breast cancer among their both close and far relatives, 10% of controls had the disease among their close relatives, 20% among their far relatives, and 16% among their family members. In Jahangardi's study, number of persons with record of disease among family members in case group was eleven, of which one, three, and seven of them were pointed to mother, aunt and other relatives, respectively, that was not statistically significant (Jahangiri L , 2007). In Banaian's study, 1.5% of participants pointed to have disease among their family members (sister and mother) (Banaian , 2005) and a meaningful relationship was observed between mean scores of attitude and perceived severity before intervention as well as scores of perceived severity with house owning situation after intervention in control group ($p<0.001$). However, no groups showed meaningful relationship with house owning situation in our study.The results of current study showed that the training program designed for female staffs of Islamic Azad University-Tonekabon branch and performed during a three months period of time had a significant influence in their preventive behaviors against breast cancer. According to these results, it can be stated that training programs can improve the preventive behaviors toward breast cancer.

A training program was designed based on heath belief model to evaluate the efficacy and efficiency of this model in improving preventative behaviors toward breast cancer. The study conducted on female staffs who were working in a university in northern Iran. The results of our study showed that this program had a positive influence on knowledge, attitude and performance of women regarding prevention of breast cancer. It seems that publicizing of this program will decrease the costs of public health in a country like Iran.

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MORBIDITY AFTER CHIN GRAFT HARVESTING USING PIEZOSURGERY VERSUS CONVENTIONAL OSTEOTOMY TECHNIQUES

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ABSTRACT: Augmentation of the resorbed site using chin bone graft is a common method. However, it involves donor site morbidity. Chin graft morbidity involves impaired sensibility in the mandibular anterior teeth, the gingival and chin–lower lip area postoperatively. The aim of this study was to investigate the morbidity at the donor site following harvest of chin bone using piezosurgery versus conventional bone cutting tools. In addition, distances between the bone harvest defect and anatomical structures such as apices of lower anterior, premolars and mental foramina were analyzed. A group of 20 patients (16 men and 4 women) who had undergone chin bone harvesting for alveolar ridge augmentation or sinus floor elevation procedures were selected. Patients were equally divided into two groups according to the cutting tools used for chin graft harvesting. Group I *(piezosurgery group)*; piezosurgery was used for chin graft harvesting. Group II *(conventional group)*; conventional cutting tools used for chin graft harvesting. The true distances between the donor defect and the apices of the adjacent mandibular teeth were measured on a digital panoramic radiograph. The distance from the lateral margins of the donor defect to the right and left mental foramina (RMF and LMF) was recorded. Sensibility of the lip, teeth, and gingiva was registered one week, one, three, six and 12 months post-operatively. A total of six (30%) patients experienced anaesthesia of the gingivae immediately postoperatively and had full recovery when reviewed at 3 month follow up. Three (15%) of these patients experienced paraesthesia of the chin and lower lip, however full recovery had occurred at the 6- month follow-up. None of the patients reported altered contour or change in profile of the chin area as well as dehiscence of the incision. Three patients experienced pain at the graft site for up to three months postoperatively. Nine patients showed a negative pulp sensitivity reaction in their lower teeth to ethyl chloride at one week postoperatively; this further improved to 5 patients at the 6-month follow-up and to 2 patients at the 12-month follow-up. Parameters comparisons between both piezosurgery and conventional groups showed no statistically significant difference. However, parameters comparisons across each group showed statistically significant difference between different parameters in teeth with and without negative response to thermal pulp testing regarding mean volume of the chin bone defect (P-value < 0.001), mean distance of the defect to mental foramen of both sides (P-value < 0.05). In conclusion, this study indicated that chin graft donor site showed some post-operative morbidity. The most frequent disturbance was impaired teeth sensibility. The lower lip and chin region showed fewer disturbances. However, by correctly placing mucoperiosteal incisions and bone cuts, the risk of sensitivity changes can be minimized. Surgical control for the piezosurgery was easier than conventional methods for mobilizing a chin bone block graft. The force necessary to produce a cut was much less compared to rotational burs. Increased temperature during bone cutting with piezosurgery was avoided which reduces the risk of bone damage as a result of overheating.
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KEY WORDS: autogenous bone, chin bone graft, morbidity, piezosurgery, bone augmentations, sinus floor elevation

1. INTRODUCTION

In some patients implant treatment would not possible without bone augmentation because of insufficient bone volume at the planned implant site. A well-documented surgical procedures for ridge augmentation comprises the application of a block graft, either with membrane coverage (guided bone regeneration), Buser et al.,(1996); Antoun et al.,(2001); Raghoebar et al.,(2007) or without membrane therapy, Raghoebar et al.,(1996); Widmark et al.,(1997); Sethi, and Kaus (2001); McCarthy et al., (2003a). In sinus floor elevation procedures, autogenous bone particles with or without bone substitutes are used to optimize the bone volume prior to or in conjunction with implant placement, Lorenzettiet et al., (1998); Yildirim et al., (2001); McCarthy et al., (2003b); Merkx et al., (2003).

Autogenous bone grafts are available from extra and intra-oral donor sites, Tolman (1995); Misch (1997). Extra-oral donor sites include iliac crest, calvarium, tibia, ribs and intra-oral sites include

maxilla, mandible and zygoma. The choice of donor site is dependent upon the quantity and quality of bone required, access to the donor site and time required for bone harvesting procedures, Misch et al., (1992); Raghoebar et al., (1996); Misch (1997). The mandibular symphysis is a favourable donor site because it is generally assumed that it has an excellent risk-benefit ratio, Triplett and Sihow (1998). Convenient surgical access, proximity of donor and recipient sites, low morbidity, and availability of larger quantities of bone over other donor sites, benefit of using intra-membranous bone versus endochondral bone, embryonic origin means early vascularisation and minimal resorption, minimal patient concern for altered facial contour and minimal discomfort are some advantages of this procedure over other intraoral sites, Gapski et al.,(2001);Joshi(2004).

Following harvesting of symphysis bone grafts, some neurosensory disturbances as altered sensation and sensitivity of lower anterior teeth and intra oral scarring have been described, Nkenke et al., (2001); Raghoebar et al., (2001); Joshi (2004); Von Arx et al.,(2005). In a prospective study evaluating intraoral donor sites for bone grafting, it was reported that 29% of symphysis graft patients had altered sensitivity of their mandibular incisor teeth. All findings were resolved within six months, Misch (1997).

Common techniques to evaluate post harvesting sequelae include pulp sensitivity test using carbon dioxide (CO_2) snow, Nkenke et al.,(2001);Von Arx et al.,(2005) and ethyl chloride sprayed onto a small cotton wool pledget, Joshi (2004) and skin sensitivity examinations such as pointed-blunt discrimination, two-point discrimination, Nkenke et al.,(2001);Von Arx et al.,(2005) and fine touch using graded suture material, Blackburn (1990); Joshi(2004) and assessment of pain using the pin-prick sensation, Mason (1988).

When using conventional bone cutting instruments such as reciprocating micro-saws, surgical burs, or trephines, there is a risk of postoperative necrosis. Comparative histological studies have shown necrosis on cut bone caused by surgical burs, trephines and micro-saws, Aro et al., (1981). Over the past eight years, piezosurgery (PS) or piezoelectric surgery (PES) has been introduced as a new technique for osteotomy and osteoplasty using ultrasonic micro-vibration. The piezosurgery blades allow for maximum intra-operative precision, minimal tissue damage, and the selective frequency of the scalpel minimizes the risk to adjacent soft tissues, Eggers et al. (2004). Ideally to cut soft tissue a frequency of 50 KHz is needed whereas Piezosurgery® device uses a frequency of 25-30 KHz at which only bone is cut. The device when used as recommended would not cut nerves, periosteum or the Schneiderian membrane, Stübinger et al., (2005); Schlee et al., (2006).

Moreover, a study conducted on a series of 100 consecutive cases to evaluate and monitor Schneiderian membrane perforation rate during sinus elevation using PS. The result of such study showed that the sinus membrane perforation rate using PS was decreased from 30% with rotary instrumentation to 7%, Wallace et al., (2007). The low pressure applied to the instrument enables a precise cut, and the selective cut characteristically protects the sinus membrane in sinus floor elevations, Barone et al., (2008). In the lower jaw, PS has been successfully used to mobilize the inferior alveolar nerve, Sakkas et al., (2008). Histological studies conducted with Sohn et al., (2004), Chiriac et al., (2005) and Happe (2007), showed minimal cellular damage to the resected bone margins, bony matrix and underlying marrow spaces. Another study conducted with Vercellotti et al., (2005) reported that PS provided more favourable osseous repair and remodelling than with conventional surgical burs with surgical ostectomy and osteoplasty procedures.

Therefore, the aim of this study was to investigate the morbidity at the donor site following chin bone harvesting using piezosurgery versus conventional bone cutting techniques. In addition, distances between the bone harvest defect and anatomical structures; apices of lower anterior and premolar teeth and mental foramina of both sides were analyzed.

2. MATERIALS AND METHODS

2.1. Materials:

2.1.1. Subjects:

Twenty patients scheduled for chin bone harvesting for alveolar ridge augmentation or sinus floor elevation procedures were selected for the current study. Indications for bone harvesting included lateral ridge augmentation in 9 patients and sinus floor elevation in 11 patients. The patients were selected from the Outpatient Clinic, Department of Oral and Maxillofacial Surgery, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt. Sixteen patients were males and 4 were females. The average age was 41 years (range 30 -55 years). Patients signed informed consent documents after they had been fully notified about the surgical procedures. All surgeries were performed under general anesthesia.

Basic evaluation included medical history, smoking habit, extra-oral and intra-oral examination, and radiographic investigation. As a first step, the recipient site was analyzed to determine the amount of autogenous bone to be harvested from the symphysis. The mandibular symphysis area was preoperatively estimated on a panoramic radiograph to be sufficient in height for bone harvesting. Pulp sensitivity of mandibular incisors/canines/first premolars teeth were preoperatively assessed by recording the response of these teeth on cold sensation using a spherical dental cotton on which an ethyl chloride vapour was sprayed, Raghoebar et al.,(1990).

The selected patients were equally divided into two groups according the bone cutting tools used for chin graft harvesting. *Group I (piezosurgery group);* piezosurgery was used for chin graft harvesting. *Group II (conventional group)*; conventional bone cutting surgical fissure burs used for chin graft harvesting.

2.2. Methods:

2.2.1. Surgical procedure

With the lip drawn anteriorly, the soft tissues were placed under tension and the incision was made in two layers, firstly through mucosa and then through the muscle and periosteal layers. A full thickness mucosal flap was raised following a vestibular incision in the inter-canine region 5 mm below the muco-gingival junction. Periosteal elevation is performed to the inferior border of the mandible to expose the symphysis. Caution was exercised to avoid the branches of the mental nerve. The mental foramina were not exposed. The location of the apices of lower incisors and canines were estimated from the panoramic radiograph. The length of the tooth was transferred to the clinical situation using a periodontal probe. After the apices of the lower incisors and canines were identified, the initial horizontal cut into the bone was made 5mm apical to the apices of the incisors, Von Arx et al.,(2005). The surface outline of the bone block was completed with a small round bur. The aim was to harvest a cortico-cancellous bone block. The lower margin of the mandible was always preserved to avoid changes in the chin contour, Nkenke et al., (2001).

In group I *(piezosurgery group)*, the osseous cuts were made with the piezosurgery inserts (*VarioSurg, NSK, Japan*). Copious isotonic saline irrigant was used to counter heat generation produced by the blade inserts. Osteotomies started using the VarioSurg Multiple impacts SG4 or SG1 insert and then performing the vestibular corticotomies using VarioSurg Multiple impacts SG1 and SG8 inserts. Osteotomy to the deep surface of the cortico-cancellous chin bone block using the double elbow-shaped VarioSurg Multiple impacts SG 14R and SG 14L inserts (Figure 1).

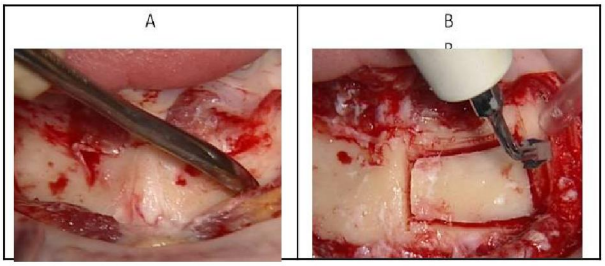

Figure (1): Photographs showing chin bone harvesting *in group I (piezosurgery group)*. A) A full thickness flap was raised following a vestibular incision in the inter-canine region 5 mm below the muco-gingival junction and B) The osseous cuts were made and completed with the piezosurgery inserts.

A check was made to ensure that the cut was through the cortical bone and into the cancellous bone. The cortico-cancellous block was then carefully mobilized using a curved bone chisel. Further cancellous bone chips were harvested from the chin with curettes or curved chisels, but the lingual cortex of the symphysis was never perforated.

In group II *(conventional group)*, the osseous cuts were made with a surgical fissure burs under copious irrigation with saline. A cortico-cancellous bone block was harvested with the aid of a 3 mm osteotome *(Frios Kit for bone grafting, Frident, USA)* which was tapped into the outlined area with a mallet (Figures 2 and 3). The surgical assistant provided support to the chin during the tapping phase. Further cancellous bone was harvested with curettes or curved chisels, but the lingual cortex of the symphysis was never perforated.

Then, the harvested bone was preserved in cold saline solution (4^0C), prepared for ridge augmentation or further chipped using a bone mill for sinus floor elevation procedures (Figure 4).

The donor site was packed with saline soaked gauze. The bone defect of the donor site was registered with three-dimensional measurements, using a caliper (width W= mesio/ distal dimension, height H= apico/coronal dimension, depth D= oro/facial dimension). Prior to soft tissue closure of the mandibular donor site, the area was copiously irrigated with saline. A gelatin sponge was applied into the donor area as a haemostatic dressing.

The chin wound was then sutured in two layers. First the mentalis was sutured with internal interrupted sutures and then the overlying mucosa was closed with a 5/0 resorbable suture. A pressure dressing (elastic tape) was applied to the chin and maintained for 5 days to minimize postoperative

swelling and formation of a hematoma. Postoperative medication included antibiotics; Augmentin, 1gm/12 hours for 6 days (875mg amoxicillin and 125 mg clavulanate potassium, GlaxoSmithKline S.A.E, El Salam city, Cairo, Egypt), analgesics; Declofenac Potassium 50 mg/ 8 hours (Cataflam, produced by novartis pharma, Cairo, Egypt), and 0.2% chlorahexidine gluconate mouth rinse, 3 times/day (Hexitol mouth wash, Arab drug Co. Cairo, Egypt) for the next 10 postoperative days.

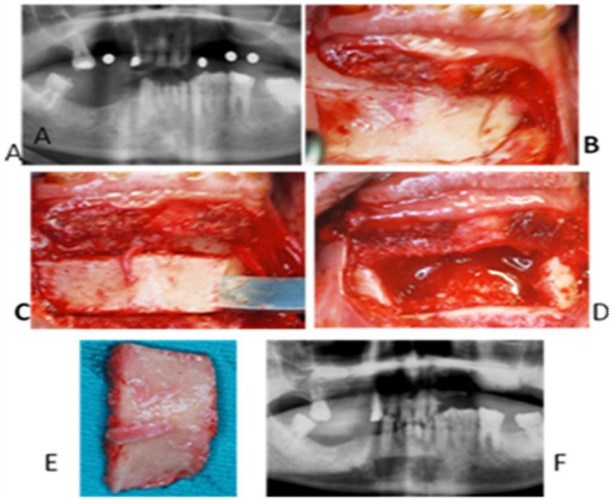

Figure (2): Photographs showing one case of chin bone harvesting *in group II (conventional group)*, A) Preoperative panoramic radiograph, B) the bone block was outlined with a small round bur, C) the osseous cuts were completed with 3 mm osteotome, D) Bone defect after chin bone harvesting, E) the harvested cortico-cancellous bone block and F) Size of bone defect after chin bone harvesting were seen in postoperative panoramic radiograph.

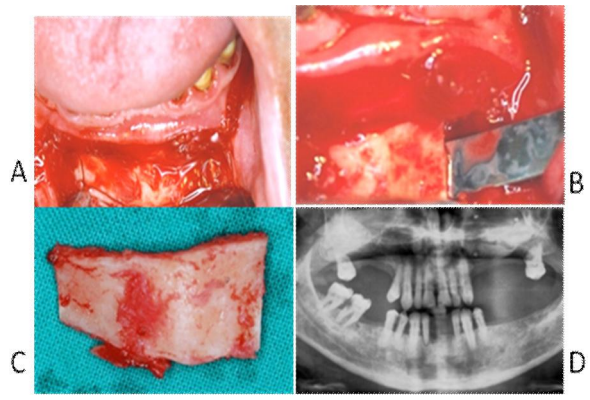

Figure (3): Photographs showing another case of chin bone harvesting *in group II (conventional group)*, A) A full thickness flap was raised following a vestibular incision in the inter-canine region and the bone block was outlined and the osseous cuts were completed with osteotome, C) the harvested cortico-cancellous bone block and D) the resulting bone defect after chin bone harvesting were seen in postoperative panoramic radiograph.

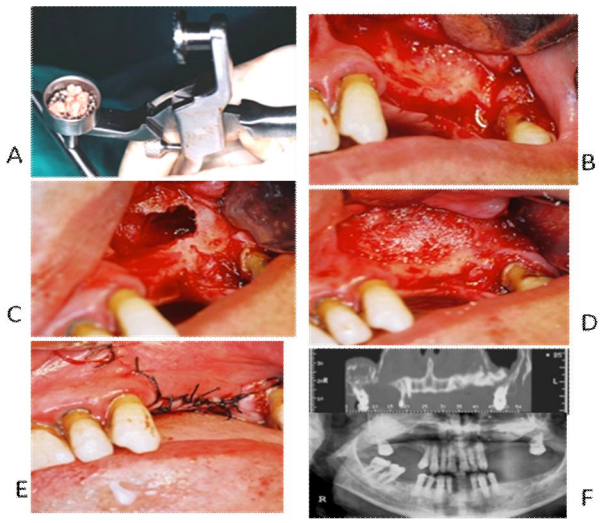

Figure (4): Photographs showing a case of sinus floor elevation, A) the harvested cortico-cancellous chin bone block was chipped using a bone mill, B) A full thickness flap was raised, C) Prepared lateral window for sinus floor elevation, D) elevated sinus floor packed with the chipped bone, E) closure of the flap, and F) grafted maxillary sinus were seen in postoperative CT scan (Dentascan) and panoramic radiograph.

2.2.2. Post-operative evaluation

All the patients were examined pre-operatively and one week, one, three, six and 12 months post-operatively. One week postoperative, the donor site was examined for the contour of the chin and lower lip. Pulp sensitivity testing was done by recording the response of the mandibular incisors, canine and first premolars on cold sensation using a spherical dental cotton on which an ethyl chloride vapour was sprayed, Raghoebar et al., (2001). Tactile sensibility or skin sensitivity of the chin–lip area was tested, Raghoebar et al., (2007) by lightly brushing the skin with a wisp of cotton (the subject should be able to count the number of contacts with the eyes closed). Superficial pain was tested, Mason (1988); Joshi (2004); Raghoebar et al., (2007) with a needle (the subject should be able to tell whether contact with the skin was made with a sharp or dull instrument with the eyes closed). Moreover, patients were asked whether they experienced an altered sensation of their gingiva, in the mucosa and skin region innervated by the mental nerve Raghoebar et al., (2007). The outcome was classified according to Raghoebar et al.,(2001) and Joshi (2004) into the following definitions; when a patient reported diminished sensation without the presence of pain then this was defined as paraesthesia. Anaesthesia was related to complete absence of sensation and dysaesthesia was characterized by an altered sensation with discomfort and pain.

The true distances between the donor defect and the apices of the adjacent mandibular teeth were

measured, Von Arx et al., (2005) on a digital panoramic radiograph (1/1 with no magnification). The distance from the lateral margins of the donor defect to the right and left mental foramina *(RMF and LMF)* was also measured. Patients were recalled again one, three, six and 12 months after the initial surgery. Pulp sensitivity testing was repeated. Neurosensory disturbances of the chin, lower lip, and alveolar mucosa and other sensory disturbances of the teeth were also assessed.

2.2.3. Statistical analysis

Data were presented as frequencies, percentages, mean and standard deviation (SD) values. Mann-Whitney U test was used for comparisons between two groups. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with PASW Statistics 18.0 (Predictive Analytics Soft Ware – SPSS; IBM Company, Chicago, IL, USA.) for Windows.

3. RESULTS

Post-operative morbidity was recorded in the patients of the current study. A total of six (30%) patients *(2 patients from group I "piezosurgery group" and 4 patients from group II "conventional group")* experienced anesthesia of the gingivae immediately postoperatively and had full recovery when reviewed at 3-month follow up. Three (15%) of these patients *(one patient from piezosurgery group and two patients from conventional group)* experienced paraesthesia of the chin and lower lip, however full recovery had occurred at the 6- month follow-up. None of the patients reported altered contour or change in profile of the chin area as well as dehiscence of the incision. Three patients *(one patient from piezosurgery group and 2 patients from conventional group)* experienced pain at the graft site for up to three months postoperatively. Nine patients *(4 patients from piezosurgery group and 5 patients from conventional group)* showed a negative pulp sensitivity reaction in their lower teeth to ethyl chloride at one week postoperatively; this further improved to 5 patients *(2 patients in piezosurgery group and 3 patients in conventional group)* at the 6-month follow-up and to 2 patients *(one patient in each group)* at the 12-month follow-up.

The average size of the chin bone donor defects as measured intra operatively was 898mm^3 in piezosurgery group and 892mm^3 in conventional group. The bone defects had a mean width of 18.1mm in piezosurgery group and 18.0 mm in conventional group, a mean height of 8.5mm in piezosurgery group and 8.6 mm in conventional group, and a mean depth of 5.4mm in piezosurgery group and 5.5 mm in conventional group (Table 1).

Mean distances measured on digital panoramic radiographs between the mental foramen and the chin bone defects measured 13mm in piezosurgery group and 12.3mm in conventional group on the right side and 11.9mm in piezosurgery group and 11.1mm in conventional group on the left side (Table 2).

Table (1): Intra-operative size of the chin bone defect

Patients' groups	Mean ± SD	Maximum	Minimum
Group I (piezosurgery group, (n=10)			
W, width *(mm)*	18.1± 5.7	26	10
H, height *(mm)*	8.5 ± 1.1	8	6
D, depth *(mm)*	5.4± 1.4	7	4
Volume (W×H×D) *(mm3)*	894± 420	1540.5	363.7
Group II (conventional group), (n=10)			
W, width *(mm)*	18.0± 5.3	24	10
H, height *(mm)*	8.6 ± 1.1	9	6
D, depth *(mm)*	5.5± 1.4	7	5
Volume (W×H×D) *(mm3)*	892± 346	1463.7	423

Table (2): Recorded distances of bone defect to adjacent mental foramina*

Patient's groups	Mean ± SD	Maximum	Minimum
Group I (piezosurgery group, (n=10)			
RMF , right mental foramen *(mm)*	13± 5.0	20	5
LMF , left mental foramen *(mm)*	11.9± 4.5	19	5
Group II (conventional group), (n=10)			
RMF , right mental foramen *(mm)*	12.3± 3.8	16	6
LMF , left mental foramen *(mm)*	11.1± 3.4	15	5

**True measurements were taken from digital panoramic radiographs in mm.*

Mean distances between the apices of mandibular first premolars/canines/incisors and the donor chin bone defect ranged from 6.6 to 9.7mm in piezosurgery group and 6.1 to 10.1mm in conventional group (Table 3).

Pulp sensitivity changes of the mandibular first premolars/canines/incisors were examined with ethyl chloride and were broken down per tooth (Table 4 in piezosurgery group) and (Table 5 in conventional group) and per patient (Table 6 in both groups). Total number of teeth in both groups was less than

160, since 36 teeth *(15 teeth in piezosurgery group and 21teeh in conventional group)* with root-canal treatment, negative thermal testing at initial examination, or missing teeth was excluded for analysis of sensitivity changes over time. 16.9% *(in piezosurgery group)* and 20.3% *(in conventional group)* of previously positive teeth had a negative reaction to ethyl chloride at one week postoperatively; this further improved to 6.1% *(in piezosurgery group)* and 8.4% *(in conventional group)* at the 6-month follow-up and to 1.5% *(in piezosurgery group)* and 1.6% *(in conventional group)* at the 12- month interval.

Figures per patient were 40%, 20%, and 10%, respectively in piezosurgery group and 50%, 30%, and 10%, respectively in conventional group. Two patients (*one patient in each group*) at 12 months post-operatively, their lower lateral incisor tooth did not react to thermal testing.

The mean distance between lower teeth apices and chin bone donor defect for teeth with negative response to thermal pulp testing was 7.5mm *(piezosurgery group)* and 7.8mm *(conventional group)* compared with a mean distance of 8mm *(piezosurgery group)* and 8.3mm *(conventional group)* in teeth normal response to thermal pulp testing (Tables 7 and 8). Corresponding data of chin bone donor defect sizes and distances to right and left mental foramina for both groups were shown in (Tables 9 and 10).

Table (3): Recorded distances of bone defect to apices of adjacent lower teeth*

Patients' groups	Mean ± SD	Maximum	Minimum
Group I (piezosurgery group, n=10)			
Right first premolar *(mm)*	9.7±2.2	14	9
Right canine *(mm)*	8.7 ±1.8	12	8
Right lateral incisor *(mm)*	6.8±3.5	11	4
Right central incisor *(mm)*	7.4±3.8	13	4
Left central incisor *(mm)*	6.6±2.9	14	5
Left lateral incisor *(mm)*	6.6±3.1	13	5
Left canine *(mm)*	8.8±3.1	14	6
Left first premolar *(mm)*	9.2±3	15	10
Group II (conventional group, n=10)			
Right first premolar *(mm)*	9.8±1.9	14	10
Right canine *(mm)*	8.7±2.3	13	6
Right lateral incisor *(mm)*	6.8±3.2	15	5
Right central incisor *(mm)*	6.9±3.5	11	4
Left central incisor *(mm)*	6.1±2.6	10	5
Left lateral incisor *(mm)*	6.2±2.8	11	4
Left canine *(mm)*	8.4±2.2	12	7
Left first premolar *(mm)*	10.1±2.6	16	9

**True measurements were taken from digital panoramic radiographs in mm.*

Table (4): Changes in pulp sensitivity of mandibular anterior teeth and first premolars in *group I (piezosurgery group)* following chin bone harvesting per tooth (*n = 80*)

Mandibular tooth	One week	One month postoperative	3 months postoperative	6 months postoperative	12 months postoperative
Right first premolar *(n = 8)*	-	-	-	-	-
Right canine *(n = 9)*	2 (22.2%)	2 (22.2%)	2 (22.2%)	1 (11.1%)	-
Right lateral incisor *(n = 9)*	1 (11.1%)	1 (11.1%)	1 (11.1%)	1 (11.1%)	1 (11.1%)
Right central incisor *(n = 8)*	2 (25%)	2 (25%)	1 (12.5%)	-	-
Left central incisor *(n = 7)*	2 (28.5%)	2 (28.5%)	2 (28.5%)	1 (14.2%)	
Left lateral incisor *(n = 8)*	1 (12.5%)	1 (12.5%)	1 (12.5%)	1 (12.5%)	-
Left canine *(n = 9)*	2 (22.2%)	2 (22.2%)	1 (11.1%)	-	-
Left first premolar *(n = 7)*	1 (14.2%)	1 (14.2%)	1 (14.2%)	-	-
Total number *(n = 65)**	11 (16.9%)	11 (16.9%)	9 (13.8%)	4 (6.1%)	1 (1.5%)

Total number of teeth less than 80, since 15 teeth with root-canal treatment, negative thermal testing at initial examination, or missing teeth was excluded for analysis of sensitivity changes over time.

Table (5): Changes in pulp sensitivity of mandibular anterior and first premolars in *group II (conventional group)* following chin bone harvesting per tooth (*n = 80)*

Mandibular tooth	One week postoperative	One month postoperative	3months postoperative	6 months postoperative	12 months postoperative
Right first premolar *(n = 7)*	-	-	-	-	-
Right canine *(n = 8)*	2 (25%)	2 (25%)	2 (25%)	1 (12.5.1%)	-
Right lateral incisor *(n = 8)*	2 (25%)	2 (25%)	2 (25%)	1 (12.5.1%)	-
Right central incisor *(n = 7)*	2 (28.5%)	2 (28.5%)	2 (28.5%)	1 (14.2%)	-
Left central incisor *(n = 7)*	2 (28.5%)	2 (28.5%)	2 (28.5%)	1 (14.2%)	
Left lateral incisor *(n = 6)*	1 (16.6%)	1 (16.6%)	1 (16.6%)	1 (16.6%)	1 (16.6%)
Left canine *(n = 9)*	2 (22.2%)	2 (22.2%)	1 (11.1%)	-	-
Left first premolar *(n = 7)*	1 (14.2%)	1 (14.2%)	1 (14.2%)	-	-
Total number *(n = 59)**	12 (20.3%)	12 (20.3%)	11(18.6%)	5 (8.4%)	1 (1.6%)

**Total number of teeth less than 80, since 21 teeth with root-canal treatment, negative thermal testing at initial examination, or missing teeth was excluded for analysis of sensitivity changes over time*

Table (6): Changes in pulp sensitivity of mandibular anterior teeth and first premolars in *group I (piezosurgery group)* and *group II (conventional group)* following chin bone harvesting per patient

Patients' groups	One week postoperative	One month postoperative	3 months postoperative	6 months postoperative	12 months postoperative
Group I, *(n=10)*	4 (40%)	4 (40%)	3 (30%)	2 (20%)	1(10%)
Group II, *(n=10)*	5 (50%)	5 (50%)	3 (30%)	3 (30%)	1(10%)
Total *(n = 20)*	9 (45%)	9 (45%)	6 (30%)	5 (25%)	2 (10%)

Table (7): Mean and Standard Deviation (SD) of distance between chin bone defect and apices of lower anterior and first premolar teeth with and without negative response to thermal pulp testing in both piezosurgery and conventional groups *(n = 124, originally 160)***

	Teeth with negative response to thermal pulp testing			Teeth without negative response to thermal pulp testing		
	Piezosurgery group n = 11	Conventional group n = 12	P-value*	Piezosurgery group n = 54	Conventional group n = 47	P-value*
Mean distance to the apex in mm ± SD	7.5±2.9	7.8±2.5	P=0.936	8±3.2	8.3±3.3	P=0.957

**Mann-Whitney U test was used for comparisons between two groups. The significance level was set at $P \leq 0.05$.*
***Total number of teeth in both groups less than 160, since 36 teeth (15 in piezosurgery group and 21 in conventional group) with root-canal treatment, negative thermal testing at initial examination, or missing teeth was excluded for analysis of sensitivity changes over time.*

Table (8): Mean and Standard Deviation (SD) of distance between chin bone defect and apices of lower anterior and first premolar teeth with and without negative response to thermal pulp testing in each group *(n for each group originally 80)***

	Piezosurgery group			Conventional group		
	Teeth with negative response n = 11	Teeth without negative response n = 54	P-value*	Teeth with negative response n = 12	Teeth without negative response n = 47	P-value*
Mean distance to the apex in mm ± SD	7.5±2.9	8±3.2	P=0.886	7.8±2.5	8.3±3.3	P=0.890

**Mann-Whitney U test was used for comparisons between two groups. The significance level was set at $P \leq 0.05$.*
*** Total number of teeth in each group less than 80, since 15teeth in piezosurgery group and 21 teeth in conventional group with root-canal treatment, negative thermal testing at initial examination, or missing teeth was excluded for analysis of sensitivity changes over time.*

Table (9): Mean and Standard Deviation (SD) of bone defect volume (size) and distances of defect to right and left mental foramen in patients with and without negative response to thermal pulp testing in both piezosurgery and conventional groups *(n of patients in both groups = 20)*

	Patients with negative response to thermal pulp testing			Patients without negative response to thermal pulp testing		
	Piezosurgery group n = 4	Conventional group n = 5	P-value*	Piezosurgery group n = 6	Conventional group n = 5	P-value*
Mean volume of the bone defect mm^3 ± SD	1243±198	1198±335	P=0.795	687±394	586±159	P=0.175
Mean distance of bone defect in mm to RMF ± SD	9±4.6	9.8±3.9	P= 0.887	15.5±3.5	14.8±1.1	P= 0.852
Mean distance of bone defect in mm to LMF ± SD	7.5±2.8	9.2±3.8	(P= 0.379)	13.5±2.7	13.4±1.1	P= 0.958

**Mann-Whitney U test was used for comparisons between two groups; the significance level was set at P ≤ 0.05.; RMF, right mental foramen; LMF, left mental foramen.*

Table (10): Mean and Standard Deviation (SD) of bone defect volume (size) and distances of defect to right and left mental foramen in patients with and without negative response to thermal pulp *testing* in each group *(n of patients in each group patients = 10)*

	Piezosurgery group			Conventional group		
	Patients with negative response n = 4	Patients without negative Response n = 6	P-value*	Patients with negative response n = 5	Patients without negative Response n = 5	P-value*
Mean volume of the bone defect mm^3 ± SD	1243±198	687±394	P < 0.001*	1198±335	586±159	P < 0.001*
Mean distance of bone defect in mm to RMF ± SD	9±4.6	15.5±3.5	P = 0.001*	9.8±3.9	14.8±1.1	P = 0.005*
Mean distance of bone defect in mm to LMF ± SD	7.5±2.8	13.5±2.7	P= 0.033*	9.2±3.8	13.4±1.1	P= 0.020*

**Mann-Whitney U test was used for comparisons between two groups; the significance level was set at P ≤ 0.05.; RMF, right mental foramen; LMF, left mental foramen.*

Statistical results

Parameters comparisons between both piezosurgery and conventional groups showed no statistically significant difference. However, parameters comparisons across each group showed statistically significant difference between different parameters in teeth with and without negative response to thermal pulp testing in each group.

Regarding mean distance between chin bone defect and apices of teeth with and without negative response to thermal pulp testing; there was no statistically significant difference between both groups in teeth with negative response *(P-value = 0.936)* as well as teeth without negative response *(P-value = 0.957)* to thermal pulp changes. Moreover, no statistically significant difference was found in teeth with and without negative response to thermal pulp changes in piezosurgery group *(P-value = 0.886)* or in conventional group *(P-value = 0.890)*.

Regarding mean volume of the chin harvest bone defect in teeth with and without negative response to thermal pulp testing; there was no statistically significant difference between both groups in teeth with negative response *(P-value = 0.795)* as well as teeth without negative response *(P-value = 0.175)* to thermal pulp changes. However, across piezosurgery group or conventional group, the mean volume of bone defect in teeth with negative response showed statistically significantly higher value than in teeth without negative response to thermal pulp testing *(P-value < 0.001)*.

Regarding mean distance of the defect to the right mental foramen in teeth with and without negative response to thermal pulp testing; there was no statistically significant difference between both groups in teeth with negative response *(P-value = 0.887)* as well as teeth without negative response *(P-value = 0.852)* to thermal pulp changes. However, across each group, the mean distance of the defect to the right mental foramen in teeth without negative response showed statistically significantly higher value than in teeth with negative response to thermal pulp testing; in piezosurgery group *(P-value = 0.001)* and in conventional group (*P-value = 0.005)*.

Regarding mean distance of the defect to the left mental foramen in teeth with and without negative response to thermal pulp testing; there was no statistically significant difference between both groups in teeth with negative response *(P-value = 0.379)* as well as teeth without negative response (*P*-value = 0.958) to thermal pulp changes. However, across each group, the mean distance of the defect to the left mental foramen in teeth without negative response showed statistically significantly higher value than in teeth with negative response to thermal pulp testing; in piezosurgery group *(P-value = 0.033)* and in conventional group *(P-value = 0.020)*.

4. DISCUSSION

The mandibular symphysis is the most commonly used intra-oral donor site. The morbidity can still be a problem for patients and it is important to highlight such morbidities to patients before considering intra-oral augmentation, Misch et al.,(1992).In the current study, a total of six (30%) patients from both groups experienced anesthesia of the gingivae immediately postoperatively and had full recovery when reviewed at 3 month follow up. Three of these patients (15%) experienced paraesthesia of the chin and lower lip, however full recovery had occurred at the 6- month follow-up. The results in this study compared favourably with previous studies of Raghoebar et al. (2001);Nkenke et al.,(2001) ;Joshi (2004);Von Arx et al., (2005) who investigated the postoperative morbidity following chin graft harvesting.

Joshi (2004) concluded that two (7.4%) patients experienced paraesthesia of the chin, lower lip and gingiva immediately post-operatively and had full recovery when reviewed at 3 months. Furthermore, Von Arx et al., (2005) observed lower lip hypoesthesia in one patient out of 30 patients at suture removal postoperatively, with complete resolution at the 6-month follow-up. Nkenke et al., (2001) identified five (25%) patients with hypoesthesia of the chin at the first postoperative examination using the two-point and pointed blunt discrimination tests. At the 12- month follow-up, two (10%) patients still had impairment of sensitive nerve function in the chin area. The relatively frequent finding of temporary and persisting hypoesthesia of the chin and lip areas in Nkenke et al. (2001) study was related to the extended para-marginal incision from region 35 to 45 in contrast to the current study as the incision was not extending beyond distal aspects of the mandibular canines and this might have contributed to a relatively lower incidence of altered sensitivity of the mental nerve as confirmed with the study of Gapski et al, (2001). Raghoebar et al., (2001) found that 9 patients out of 21 patients experienced paraesthesia of the chin region and only 2 patients had full recovery when reviewed after 3 months post-operative.

The most likely reason for the lip and/or chin paraesthesia or hypoesthesia is due to neuropraxia of the incisive nerve or the terminal branches of the mental nerve, Misch et al., (1992); Bavitz et al.,(1993). The incidence of temporary mental nerve paraesthesia is thought to be approximately 10% (compared with 15% in the current study) and post-operative neuropraxia is not uncommon, Misch et al., (1992). When a chin graft is harvested, the mental nerve is often stretched during flap elevation and flap retraction as well as it is important not to underestimate the distance of the inferior alveolar nerve because of the S-shaped course of the nerve before leaving the mandible, Bavitz et al., (1993). It is therefore important to assess nerve function pre-operatively and patients should be warned of the possibility of altered sensation of the gingiva, lower lip and chin region post-operatively.

Altered sensation of the lower teeth is also a common temporary post-operative symptom, Misch (1995). In the current study, a total of nine (45%) patients from both groups experienced in their lower teeth changes in pulp sensitivity with a negative reaction to ethyl chloride at one week postoperatively; this further improved to 5 (25%) patients at the 6-month follow-up and to 2 (10%) patients at the 12-month follow-up. Commonly lower central incisor teeth were affected. The result of this study was in agreement with Joshi (2004) who reported that five (18.5%) of patients experienced numbness of the lower anterior teeth at the first post-operative visit. Lower anterior incisor teeth were commonly affected. At 12 months, two patients continued to have no sensitivity in their lower anterior incisor teeth. In contrast to the findings of the current study, Nkenke et al., (2001) showed that canines were affected preferentially.

There was no statistically significant difference in the current study between both groups or within each group regarding mean distance of bone defect and apices of teeth with as well as without negative response to thermal pulp changes; means ± SD was 7.6±2.7 and 8.1±3.2 in teeth with and without negative response respectively. The data of the cited studies, Misch(1997); Hunt, and Jovanovic (1999); Cranin et al.,(2001), as well as, of the present study underline that a generally recommended safety distance of 5mm during graft harvest below the apices of lower anterior teeth does not appear to be the decisive factor whether pulp sensitivity changes are to occur or not. Animal experiments performed by Neukam et al., (1981) showed that the margin of

safety should be at least 8 mm to preserve blood supply of the front teeth.

The present study found a significant difference regarding the mean volume of the bone defect as well as mean distance of the defect to mental foramen of both sides in patients with sensitivity changes compared with patients without such changes. Therefore, the width of the donor defect as well as the distance to the mental foramina of both sides might be of influence whether pulp sensitivity changes are to occur or not. Extending the donor defect towards the mental foramen, the contents of the incisive canal (anterior extension of mandibular canal), which innervate the teeth, may be disturbed, Misch (1997). Therefore, the current study found that a panoramic radiograph was mandatory for preoperative location of the mental foramen.

Furthermore, negative pulp response of the adjacent teeth to ethyl chloride in the current study was found in 16.9% *(piezosurgery group)* and 20.3% *(conventional group)* at one week post-operative. At the 6-month follow up, 6.1% *(piezosurgery group)* and 8.4% *(conventional group)* of teeth showed negative pulp response. At 12-month follow up, 1.5% *(piezosurgery group)* and 1.6% *(conventional group)* of teeth showed negative pulp response. The findings of this study were in agreement with similar studies of Nkenke et al., (2001); Von Arx et al., (2005) reported that pulp sensitivity changes were found in 18.6% of adjacent teeth at the time of suture removal. At the 6-month follow-up, 8.1% of teeth, and at the 12-month re-examination, 0.6% of teeth presented with altered sensitivity. Nkenke et al., (2001) reported on 20 patients who underwent harvesting of chin grafts, and who were followed up for 12 months. At one-week postoperative evaluation, 22% of the examined teeth had lost their pulp sensitivity. Percentages of teeth with negative pulp sensitivity decreased to 13% at 6 months and to 11% at 12 months. In agreement with the current study, the majority of studies conducted by Chiapasco et al., (1999) and Nkenke et al., (2001) illustrated a continuous improvement of pulp sensitivity over time; however surveys of at least 12 months showed that a considerable number of teeth may present with a permanent change of pulp sensitivity.

None of the patients in the current study showed pulpal necrosis in agreement with Von Arx and Kurt (1998) who found that pulpal necrosis following chin graft harvesting was a very rare finding and might be explained by arterial anastomosis from Rami inter-incisivi of the sublingual artery to the Arteria alveolaris inferior anterior. Therefore, if the apices of the mandibular incisors and canines are not damaged during bone drilling, pulp necrosis is unlikely to occur following symphyseal bone harvesting, Van der Zypen (1985). None of the patients in the current study reported altered contour or change in profile of the chin area. This was in agreement with Joshi (2004) who observed that out of 27 patients, none of those patients complained of altered chin morphology. Cotter et al.,(2002) reported that recently grafts harvested from the mandibular lower border showed no discernible change of significance in chin morphology.

A total of three (15%) patients from both groups in the current study experienced pain at the graft site for up to three months postoperatively. This finding was in agreement with Raghoebar et al.,(2001) who reported that 9 (42.8%) patients presented with pain at chin bone defect area at suture removal postoperatively; this further improved and only weather-related discomfort was presented in 4 (19%) patients at the 3-month follow-up.

Wound dehiscence is a short-term postoperative complication following chin bone harvesting. Misch (1997) observed dehiscence of the incision line in 9.6% of the patients with alveolar mucosa incision. No dehiscence occurred following a sulcular incision. The application of an extra oral pressure dressing is recommended to prevent separation of wound margins. Von Arx and Kurt (1998) observed wound dehiscence in 20% of cases using alveolar mucosa approach. Observing these surgical approaches, no wound dehiscence was found in the current study as a sulcular incision was selected.

In conclusion, a preoperative examination should include adequate radiographs and sensibility tests of the soft tissue and teeth, presenting valuable baseline information when discussing post-surgical chin graft harvesting morbidity. Safety margins to adjacent vital structures must not be compromised, and instead alternative donor sites should be discussed when larger grafts are required. The current study found that the surgical control for the piezosurgery was easier than conventional methods for mobilizing a chin bone block graft. The force necessary to produce a cut was much less compared to rotational burs. Increased temperature during bone cutting with piezosurgery was avoided which reduces the risk of bone damage as a result of overheating. The piezosurgery osteotomy makes a narrow cut with little bone wastage. Temporary paraesthesia of the mental nerve following chin graft harvesting was a very rare finding and showed complete resolution. Thermal pulp sensitivity changes of lower anterior teeth were observed less frequently with improvement over time. Despite the above morbidities, harvesting of chin bone was successful

and providing patients with fully informed of the possible risks of altered sensation of the gingiva, lower lip and chin area along with loss of sensitivity of lower anterior teeth, autogenous bone remains the best option.

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Deformation of Circular Holes at Flanging Process of Thin Sheets

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Abstract: The theoretical analysis of flanging process of circular holes in thin plates taking into account the interconnected changes of a thickness and deformation hardening is carried out. Possibility of definition of current values of strains in non-stationary processes of forming is shown. In process of flanging the co-ordinate of the material element differentiating area of compression and a stretching in meridinal direction is defined.
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Key words: Sheet Metal Forming, Thickness Changes, Work Hardening, Flanging

1. Introduction

Numerous problems of forming sheet metal demand solutions of problems on definition of the stress-strain state at big plastic deformations. These problems primarily include the prediction of the parameters of strength and precision products manufactured by forming stamping operations. A distinctive feature of such problems is that the same source material elements of the sheet blank, strain in the field of plastic strain, acquire the characteristic features caused by these strains. The peculiarity of these elements is characterized by indicators such as change in thickness, the accumulated strain, yield stress, etc. In the process of forming a certain strain accumulates in this element and, as a result of strain hardening, the yield stress of this element changes. Consequently, the prediction parameters of strength and accuracy is possible with the known distribution of strain intensity, since, on the one hand, it is uniquely determined by the components of strain, on the other - indicates the degree of strain hardening. The given article serves as a continuation of a series of works [Nazaryan, Konstantinov, and Nazaryan, Konstantinov, Arakelyan and Nazaryan, Arakelyan] and is devoted the theoretical analysis of flanging process circular apertures in thin plates taking into account the interconnected change of thickness and strain hardening (while the known aspects of flanging process are ignored).

2. New Theory

Let us consider the process of forming when flanging circular holes in thin plates carried out with a flat end punch [Priests, Kovalev]. In papers [Nazaryan, Konstantinov, and Nazaryan, Konstantinov, Arakelyan and Nazaryan, Arakelyan] it is shown that the original equations describing the plastic plane stress condition, which occurs when flanging circular holes in thin plates, namely:

· equation of balance taking into account variability of thickness,
· equation of stress and strain increments,
· constant volume condition,
· Mises' plasticity condition

are reduced on the deviatoric plane of the plasticity cylinder to a fairly simple differential equation:

$$d\sigma_\rho = \sigma_s d\varepsilon_i. \qquad \text{(Equ. 1)}$$

In deriving (1) meridional σ_ρ and σ_θ circumferential stresses are expressed through the parameter deformed state, used as:

$$\sigma_\rho = \frac{2}{\sqrt{3}}\sigma_s \cos(\varphi + \pi/6);\ \ \sigma_\theta = -\frac{2}{\sqrt{3}}\sigma_s \sin\varphi, \qquad \text{(Equ. 2)}$$

whereas components of strain increments are presented by known dependences

$$d\varepsilon_\rho = d\varepsilon_i \cos\varphi;\ \ d\varepsilon_\theta = d\varepsilon_i \cos(\varphi + 2\pi/3)$$
$$d\varepsilon_z = d\varepsilon_i \cos(\varphi + 4\pi/3) \qquad \text{(Equ.3)}$$

which unequivocally satisfy intensity of strain increment defined taking into account condition of volume constancy with the following parity

$$d\varepsilon_i = \frac{2}{\sqrt{3}}\sqrt{d\varepsilon_\rho^2 + d\varepsilon_\rho d\varepsilon_\theta + d\varepsilon_\theta^2}. \qquad \text{(Equ. 4)}$$

The isotropic hardening is represented on the deviatoric plane of the cylinder plasticity in the form of circles with radiuce $\sigma_s/\sqrt{3}$, which increase in the radial direction by an amount determined by the value of the accumulated strain [3]

$$\sigma_s = A\varepsilon_i^n \qquad \text{(Equ. 5)}$$

where A and n are the constants characterising strain hardening. Current value of the accumulated strain, taking into account a condition of volume constancy is defined by dependence

$$\varepsilon_i = \frac{2}{\sqrt{3}}\sqrt{\varepsilon_\rho^2 + \varepsilon_\rho\varepsilon_\theta + \varepsilon_\theta^2}.$$

(Equ. 6)

From comparison (4) and (6) follows that generally intensity of strain increments $d\varepsilon_i$ is not equal to an increment of intensity $d\bar{\varepsilon}_i$ defined by differentiation of dependence (6). Therefore integration of the differential equation (1) taking into account (5) is possible only for radial directions of strains at which for the given material element the relation of strain increment components remains constant. Limits of parameter change φ in the considered problem are established proceeding from flanging process definition at which for all center current values of district deformations are positive, increasing from zero on border of non-deformable part of blank to the greatest value on aperture edge. From the analysis of dependences (2) follows that on border of non-deformable part of blank meridional stretching stresses cannot exceed value $2\sigma_s/\sqrt{3}$ corresponding to direction $\varphi = 11\pi/6$ at which $\varepsilon_\theta = 0$, and from the absence condition of meridional stretching stresses on aperture edge it follows that the boundary element is deformed under conditions of radial stretching in a positive direction of axis ε_θ that corresponds to direction $\varphi = 4\pi/3$.

From the above arguments it follows that theoretically possible range of realisation of flanging process at which for all material elements current value of circumferential strain is $\varepsilon_\theta \geq 0$, it is located on deviatorial planes of plasticity cylinder within the change of parameter φ in range $4\pi/3 \leq \varphi \leq 11\pi/6$. Hence, all types of deformations in flanging process are completely characterised by the radial beams located in sector with central angle $\varphi = \pi/2$ (fig. 1).

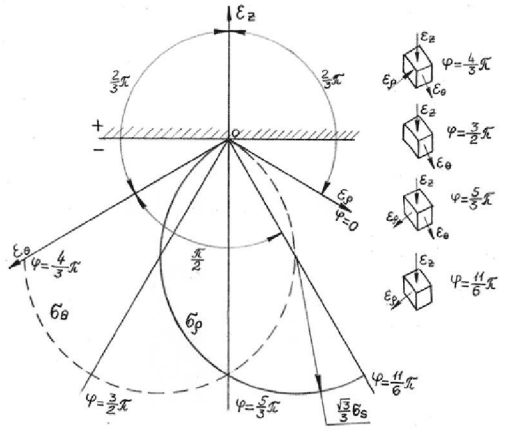

Fig.1. Stress state and strain types in flanging

If the strain direction coincides with axies ε_θ, then ε_θ is stretch strain in circular direction, and ε_ρ and ε_z are compression strains numerically equal to $\varepsilon_\theta/2$. If the strain direction coincides with the negative direction of axis ε_z, then ε_z is strain compression in thickness, and ε_ρ and ε_θ are strech strains numerically equal to $\varepsilon_z/2$. If the strain direction coincides with direction $\varepsilon_\rho = 0$, and ε_θ and ε_z are equal in value and are opposite in sign then pure shear or plane strain in plane (θ, z) takes place. If the strain direction coincides with direction $\varepsilon_\theta = 0$, and ε_ρ and ε_z are equal in value and are opposite in sign pure shift or plane strain in plane (ρ, z) takes place. Thus, strain direction with angles $\varphi = 3\pi/2$ and $11\pi/6$ correspond to pure shift, accordingly, in planes (θ, z) and (ρ, z), and with angles $\varphi = 4\pi/3$ and $5\pi/3$ - pure tension and compression, respectively, in the circumferential direction and in the direction of the thickness.

To establish a correspondence between the coordinates of the elements and strain state parameters let's integrate equation (1) i naccord with previously adopted assumption about the radial nature of the current strain accumulation. Equating the result of integrating the value of the meridional stresses, determined by the dependence (2), we obtain

$$\varepsilon_i^{1+n} - \varepsilon_{кр}^{1+n} = (1+n)\varepsilon_i^n \frac{2}{\sqrt{3}}\cos(\varphi + \pi/6)$$

(Equ. 7)

When we integrate (1) the boundary condition is used according to which the boundary element (aperture edge is deformed in the circumferential direction in a linear stretch throughout the entire process of forming).The known distribution of the strain intensity at the angle φ allows to establish the relationship between the coordinate ρ of the element and the parameter φ on the deviatoric plane of the cylinder plasticity. For the initial stage of the process of forming ($\varepsilon_{kp} = 0$), differentiating the expression (7) and equating the result (3) ($d\bar{\varepsilon}_i = d\varepsilon_i$, $d\varepsilon_\theta = d\rho/\rho$) [2], we obtain:

$$d\varepsilon_\theta = \frac{d\rho}{\rho} = (1+n)(\sin\varphi\cdot\cos\varphi + \frac{\sqrt{3}}{2}\sin^2\varphi + \frac{\sqrt{3}}{6}\cos^2\varphi)d\varphi$$

(Equ. 8)

Expression (8) after integration will become:

$$\ln\rho = (1+n)\left(\frac{\sqrt{3}}{3}\varphi - \frac{1}{4}\cos 2\varphi - \frac{\sqrt{3}}{12}\sin 2\varphi\right) + c$$

(Equ. 9)

where the constant of integration c is from the boundary condition, according to which at $\varphi = 4\pi/3$ $\rho = r_0$ (r0 is the initial radius of aperture). Substituting integration constant in (9), after simple transformationswe get:

$$\frac{\rho}{r_0} = \exp(1+n)\left(\frac{\sqrt{3}}{3}(\varphi - 4\pi/3) - \frac{1}{4}\cos 2\varphi - \frac{\sqrt{3}}{12}\sin 2\varphi\right)$$

(Equ. 10)

From (10) follows, that strain area has the greatest values at $\varphi = 11\pi/6$ ($\varepsilon_\theta = 0$), $\rho = \rho_0$ (ρ_0 - the radius of matrix die) and equals

$$\frac{\rho_0}{r_0} = \exp(1+n)\frac{\sqrt{3}}{3}\frac{\pi}{2} \approx 2{,}475\cdot(1+n)$$

(Equ.11)

At $n = 0$ the limit value of relation ρ_0/r_0 coincides with the result of work [1] which was received when solving the problem taking into account thickness change for ideally rigid plastic model of deformable material.

Dependence (10), establishing unequivocal connection between initial co-ordinate of considered element and parameter φ allows to newly consider the flanging process dividing it into two stages.

At the initial stage of strain, as the lowering of the punch takes place, the area of plastic strains is formed from the external border (at the edge of the matrix die) to te internal, with blank elements moving in the meridional direction, and their thickness decreases. Consequently, there is an element deformable in the direction $\varphi = 3\pi/2$, for which meridional strain is $\varepsilon_\rho = 0$ throughout the forming process. To define co-ordinate ρ for this element, substituting in (10) $\varphi = 3\pi/2$, we obtain

$$\frac{\rho}{r_0} = \exp(1+n)\left(\frac{\sqrt{3}}{3}\frac{\pi}{6} + \frac{1}{4}\right) \approx 1{,}737\cdot(1+n)$$

(Equ.12)

Comparing the received result for the relative co-ordinate differentiating the area of shortening from the area of lengthening in meridional direction at $n = 0$ with the result of work [4] ($\rho/r_0 \approx 2$) which is obtained without considering the change in thickness and influence of strain hardening, we see, that in [4] it is a little overestimated. Thus, all material elements with a relative co-ordinate less in value defined by (12), in are shortened in meridional direction, being exposed to radial compression, whereas elements with relative co-ordinate more in value in meridional direction are extended, being exposed to radial stretching. Hence, estimation of the flanging process parameters should be based on the flanging coefficient. At small values of flanging coefficient of deformations $\varepsilon_\rho \le 0$, the total height of the bead is less than the width of flanging part of the blank. At great values of flanging coefficient when in the strain area ε_ρ changes its sign, compressive strains in meridial direction are somewhat compensated by stretching strains, and the estimation application, which is based on equality of length sweep length of bead in the midline on an average line and the width of the flanging part of the bead. Knowing the character of strain intensity distribution , it is possible to define the strain components:

$$\varepsilon_\rho = \frac{1+n}{2}\left(1 + \cos 2\varphi - \frac{\sqrt{3}}{3}\sin 2\varphi\right),$$

$$\varepsilon_\theta = -\frac{1+n}{2}\left(\cos 2\varphi + \frac{\sqrt{3}}{3}\sin 2\varphi\right),$$

$$\varepsilon_z = -\frac{1+n}{2}\left(1 - \frac{2\sqrt{3}}{3}\sin 2\varphi\right);$$

(Equ.13)

Fig. 2 shows the changes of strain intensity and components upon the change of parameter φ in range $4\pi/3 \le \varphi \le 11\pi/6$ at the initial stage of strain ($\varepsilon_{kp} = 0$) at $n = 0.2$.

3. Result and discussion

From (13) and fig. 2 it follows, that in the strain area some element with parameter of strained state $\varphi = 19\pi/12$ during the initial moment of deformation receives the greatest circumferential strain, which equal to $\frac{\sqrt{3}}{3}(1+n)$. Relative co-ordinate of the material element which has received the greatest circumferential strain is found from (10).

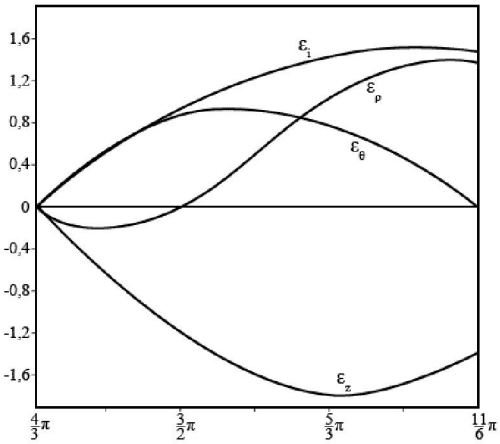

Fig. 2. Distribution of strain intensity and components at the strain initial stage of strain.

$$\rho / r_0 \approx 2{,}1 \cdot (1 + n)$$

At the second stage of strain together with the increase in current radius of aperture edge formation of a vertical wall of bead begins, and at the final moment of strain all material elements in circumferential direction receive the set size of strain defined by the relation of the sizes of an aperture and the deforming tool. On fig. 3 ways and distributions of deformations are presented at different strain values of aperture edge for parameter of strain hardening $n = 0.2$.

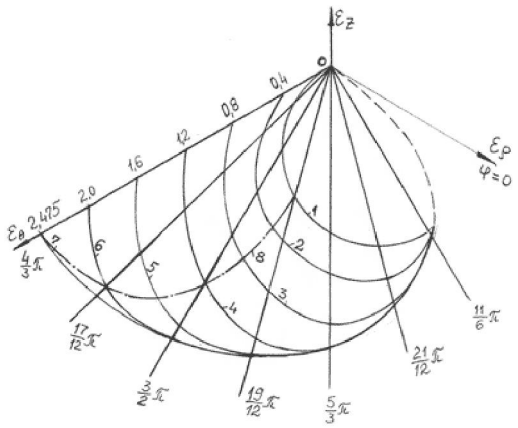

Fig. 3. Ways and distribution of deformations at the second stage of strain (curves 1 and 2-7 of strain distributions on initial and the subsequent stages of strain, directions $4\pi/3 \ldots\ldots 11\pi/6$ - ways of deformations, line 8 - line of the greatest circumferential strains.)

Given that the strain could not take zero values in leaps at the boundary of the un deformed blank ($\varphi = 11\pi/6$), on fig. by 3 dashed lines the area of smooth reduction of strain intensity from current sizes to zero is shown. At the second stage of strain forming process has strongly pronounced non-stationary character at which there is a continuous change of the values of the plastic strain area. The impossibility to define the current values of strain components from (7) in analytical form is connected with the impossibility of its representation in the form of explicit function from parametre φ and strain value of aperture edge. Therefore definition of current values and character of change strain components change is carried out by graphic imaging of vector function $\bar{\varepsilon}_i(\varphi, \varepsilon_{кр.})$ on oblique axes $\varepsilon_\rho, \varepsilon_\theta, \varepsilon_z$. Fig. 4 presents the charts of current value changes of strain components at the variable strain area for n=0.2.

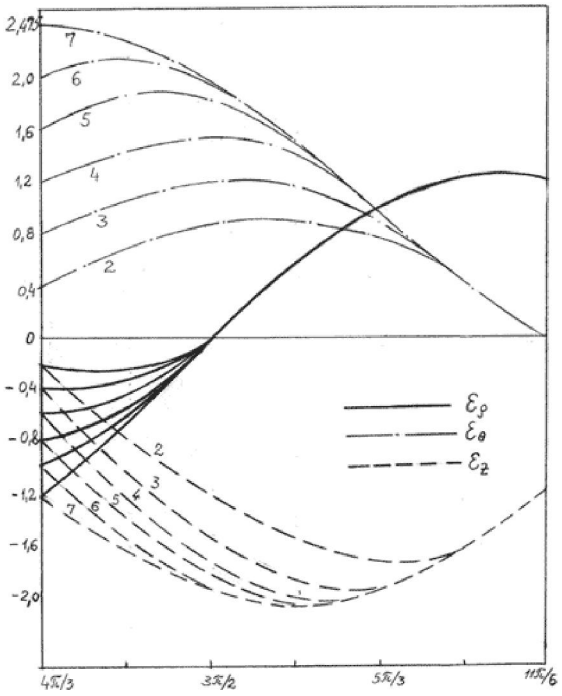

Fig. 4. Charts of Current value chamges of strain components at variable area (curves 2 - 7).

From the presented figures it follows, that for the entire area strain in thickness is negativ, whereas in circumferential it is positive. Radial strains in direction $\varphi = 3\pi/2$ change their sign as a result of which part of the area finds itself in the state of radial compression during the forming process, and part of it is in the state of biaxial stretching.

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Toxigenic fungi and aflatoxin associated to nuts in Saudi Arabia

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Abstract: A survey was carried out in July-Sept., 2009 to obtain data on the occurrence of aflatoxin and the aflatoxins-producing potential of fungi isolated from nuts (almonds, peanuts, hazelnuts, pistachio nuts, Walnut and Cashew) in region of Qassim in Saudi Arabia. The samples were analyzed for aflatoxins by immune affinity column (IAC) clean-up with liquid chromatography and fluorescence detection. Percentages of positive samples with aflatoxins were 80, 80, 60, 40, 40, 20 % for pistachio, peanuts, walnuts, almonds, hazelnuts and cashews. Concentrations of aflatoxin B_1 were ranged between (38- 45, 11- 90, 41 -90, 0.3-3.6 ,62-120 and 70-140 " µg/kg") respectively.100% of samples showed variable incidence of fungal contamination. Fungi present in samples were *Penicillium spp, Aspergillus niger*, *A. flavus* and *Rhizopus spp*. Results showed that isolates of *A. flavus* were able to produce aflatoxins B_1, B_2, G_1, and G_2. The purpose of the survey was to determine levels of aflatoxins and to monitor the effectiveness of the controls in place to limit consumer exposure to aflatoxins.

Keywords: exposure to carcinogens, aflatoxins, nuts, HPLC, Fungi, food contaminants**.**

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1. Introduction

Mycotoxins; toxic secondary metabolites of fungi are biological in origin. Despite efforts to control fungal contamination, toxigenic fungi are ubiquitous in nature and occur regularly in worldwide food supplies due to mold infestation of susceptible agricultural products, such as cereal grains, nuts, and fruits. Thousands of mycotoxins exist, but only a few present significant food safety challenges. The natural fungal flora associated with foods is dominated by three genera of fungi; *Aspergillus*, *Fusarium*, and *Penicillium,* which except for the *Fusarium* plant pathogens, may include commensals as well as pathogens. Groundnut is frequently infected with fungi that produce mycotoxins during and after harvesting, which affect the quality and safety of human food (Martin *et al*., 1999). They are toxic to humans and animals, cause significant reductions in crop yield and cause economic losses (Gourama and Bullerman, 1995; Gqaleni *et al.,* 1996). Their occurrence in various countries has been well documented (Bathnagar and Garcıa, 2001). *Aspergillus flavus* and *Aspergillus parasiticus* are important contaminants of certain foods and animal feeds because of their ability to produce aflatoxins (Farr *et al.,* 1989). When these fungi invade and grow in commodities such as peanuts, corn and cottonseed, the resulting contamination with aflatoxins often makes the commodities unfit for consumption (Vardon, 2003). Aflatoxins are considered the most carcinogenic, mutagenic and teratogenic substances found naturally in foods and feeds (Conner, 1993). These metabolites cause liver damage to humans and to most experimental animal species tested (Gradelet *et al.,* 1997). Consumption of mycotoxin-contaminated foods has been associated with several cases of human poisoning, or mycotoxicosis, sometimes resulting in death (Bathnagar and Garcıa, 2001). The International Agency for Research on Cancer (IARC) has classified aflatoxin B_1 (AFB_1) as a group I carcinogen, primarily affecting liver (IARC, 1993). Natural occurrence of AF in nuts has been studied in various countries. According to a report from Mexico, 2.2% of pistachio nut samples analyzed contained AF higher than 20 ng/g (JECFA, 1998). In Sweden, 9.5% pistachio nut samples contained AFB1 higher than 2 ng/g (Thuvander *et al.,* 2001). According to Ministry of Agriculture and Rural Affairs, Republic of Turkey, (2002) analysis of 523 pistachio nut samples in Turkey the mean of AFB1 ranged 1–3.78 ng/g and the maximum level detected was 113 ng/g.

The present study was undertaken to determine the occurrence of fungi, can production of aflatoxins in nuts in Saudi Arabia.

2. Material and Methods

Methods:

Samples:

Thirty samples of nuts (almonds, hazelnuts, pistachio nuts, Cashew ,Walnut and peanuts) were randomly collected from Saudi Arabia , Qassim region (Buraydah, Unayzah, Al-Ras, Al- Badaye and Al- Bukayriyah) during Juli to Sept at 2009.

Isolation of fungi associated with nuts.

Fungi associated with nuts were isolated according to methods of Lichtwardth *et al.*, (1958), Mislivec (1977) and Ichinoe *et al.* (1983). All nut samples were immersed in sodium hypochlorite solution (5%) as a sterilizer, for 3 minutes, rinsed 3 times in sterile distilled water then dried between sterile filter paper. Isolation of fungi was made by randomly taking 100 disinfested grains from samples of each location and directly plated in 20 Petri-dishes (5 grains per dish) on Czapek's agar medium, then incubated at 25°C for 4 days. Then, the percentage of the infected grains was determined and the fungal colonies that developed from the infected grains were counted, isolated, purified and maintained on a slant potato dextrose agar (PDA) medium for identification trials.

Identification of the fungal isolates:

All the isolated fungi were identified by studying the cultural characteristics, as well as the microsocial structures on Gzapek's agar medium, according to Harigan and Margaret (1966), and according to the procedures of Gilman (1957), Barnnett and Hunter (1972) Numbers of the species of *Aspergillus* were classified according to the key published by Raper and Fennell (1965), while for species of other isolated fungi the "Manual of clinical mycology" by Count *et al.* (1954) was used.

Production of AFs by isolated *A. flavus* strain:

The ability of AFs production by *A. flavus* strains using liquid media (YES) was investigated according to Singh *et al.,* (1991).

Aflatoxin standard:

Preparation of aflatoxin standard was carried out according to the Association of Official Analytical Chemists, A.O.A.C.(2000). Crystals of aflatoxins B_1, B_2, G_1 and G_2 were diluted using benzene-acetonitrile (98: 2 v/v) to obtain a concentration of 8 to 10 µg/ml (stock solution).

Equipment and chromatographic conditions:

Extraction procedure:

AFs were analyzed in nuts according to the method reported by Stroka, et al., (2000). Briefly, 10 g of nut samples were added to 1 g of NaCl , then blended with 40 ml of methanol/water (80:20) and 20 ml of cyclohexane for 3 min. After separation of the two phases, cyclohexane was eliminated. Extracts were filtrated on a Whatman filter paper No. 4. Immunoaffinity column clean-up An aliquot of 10 ml was diluted with 60 ml of PBS buffer (pH 7.4). An immunoaffinity column (IAC AflaTest, Vicam, USA) for AFs analysis was conditioned with 10 ml of PBS buffer by gentle syringe pressure at a flow rate of 5 ml/min. Then, the mixture of the filtrate diluted extract (70 ml) was applied to the IAC column (1–2 drops per second), followed by a washing with 20 ml of bi-distilled water and then dried with air. AFs were then slowly eluted from the IAC with 2 ml methanol into a glass vial.

Precolumn derivatization:

The eluate was evaporated to dryness with a gentle stream of N2 at 52 ºC, redissolved with 100 µl of TFA for 3 min, re-evaporated to dryness with N2 at 52□ C, and reconstituted in 500 µl of the mobile phase for LC analysis. TFA was added to AFs working standards in the same conditions as the extract samples to derivatize AFB1 , AFB2 , AFG1and AFG2.

Determination of Aflatoxins A by HPLC:

High-performance Liquid Chromatography "HPLC" (Agilent 1100 series) equipped with a fluorescence detector (G 1321A) analysis was carried out with a liquid chromatograph equipped with solvent delivery systems (Agilent Technologies, Inc. 200 Regency Forest Drive, Suite 330 Cary, NC 27511 USA) system containing a G1322A Vacuum Degasser, a G1312A Binary pump and a reverse-phase analytical column packed with C_{18} material (Agilent ZORBAX, DB- 5 µm, 150 mm × 4.6 mm) . The mobile phase consisted of water: acetonitril : methanol (240:120:40) (Deabes, 2008). Separation was performed at 40 °C temperature at a flow rate of 1.0 ml min-1; the injection volume was 50 µl for both standard solutions and sample extracts by auto sampler (G1329A). The detection was performed using fluorescence detector was operated at an excitation wave length of 360 nm and an emission wave length of 440 nm .

Quantitation*:* The mixed solutions of standard as well as sample extract after derivatisation were filtered through a 0.22 µm membrane filter and loaded (50 µl) into autosampler. The elution order of the four aflatoxins was G_2, B_2, G_{2a} (G_1 derivative), B_{2a} (B_1 derivative). AFs contents in samples were calculated from chromatographic peak areas using the standard curve.

3. Results and Discussion:

Isolation and identification of fungi associated with nuts:

It is well known that some fungi grow on/ and in nuts and these fungi can deteriorate the stored nuts. Therefore, the present work, was started by isolation and identification such fungal species. Isolation of fungi was made by using Czapek's agar as growing media.

Fungi isolation on Czapek's agar medium:

Data in Table (1) represent the fungi associated with Czapek's agar medium from nuts purchased from retail markets in Qassim, Saudi Arabia during the summer season of 2009.

Table (1): Fungi associated with nut samples collected from Qassim region Saudi Arabia during 2009.

Commodities	No of samples	% of Infections	Total fungal Counts	Isolated Fungi		
				Aspergilluss	*Penicillum*	*Rhizopus*
Almond	**5**	**100**	**120**	**57.00**	**40.00**	**3.00**
Pistachio	**5**	**100**	**153**	**60.00**	**32.00**	**8.00**
Hazelnut	**5**	**100**	**140**	**53.00**	**33.00**	**14.00**
Cashew	**5**	**100**	**60**	**50.00**	**40.00**	**10.00**
Walnuts	**5**	**100**	**160**	**57.00**	**33.00**	**10.00**
Peanuts	**5**	**100**	**163**	**67.00**	**24.00**	**9.00**

Table (2): Toxicity of *Aspergillus flavus* isolated from nuts and the ability for aflatoxins production in YES medium.

Kind of Nuts	Isolates of *Aspergillus* tested (No.)	Isolates of *A. flavus* (No.)	Isolates of *A .flavus* producing Aflatoxins (No.)	Isolates of *A. flavus* producing Aflatoxins (%)	Total Aflatoxins concentration mg/l	
					Min	Max
Almonds	40	25	22	88	20	40
Pistachio	60	42	33	78	12	60
Hazelnuts	65	32	27	84	16	50
Cashews	30	15	7	46	1.2	6
Walnuts	70	50	40	80	30	70
Peanuts	90	62	58	93	65	150

Table (3): Survey of aflatoxins in nuts collected from Qassim, Saudi Arabia during summer , 2009

Kind of nuts	No. of samples	No. of positive samples	% of positive samples	Aflatoxins concentration µg/kg							
				AFB1		AFB2		AFG1		AFG2	
				Min	Max	Min	Max	Min	Max	Min	Max
Almonds	5	2	40	38.00	45.00	11.00	13.00	0.00	2.30	0.00	4.20
Pistachio	5	4	80	11.00	90.00	2.00	70.00	1.10	55.00	1.90	25.00
Hazelnuts	5	2	40	41.00	55.00	12.90	18.00	3.10	6.00	5.20	8.40
Cashews	5	1	20	0.30	3.60	0.10	2.30	0.00	0.00	0.00	0.00
Walnuts	5	3	60	62.00	120.00	13.00	33.00	5.30	11.00	7.90	17.00
Peanuts	5	4	80	70.00	140.00	18.00	71.00	2.20	10.10	3.10	13.40

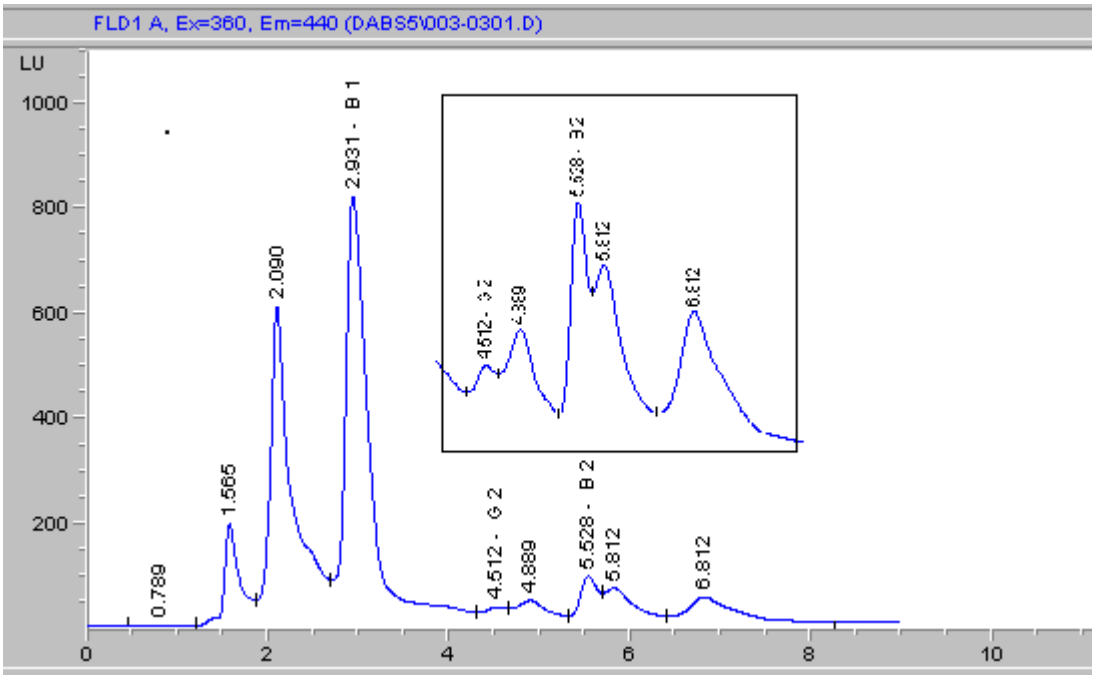

Figure (1): High Performance Liquid Chromatogram of a positive sample of aflatoxins (AFG_1,AFB_1, AFG_2 and AFB_2)

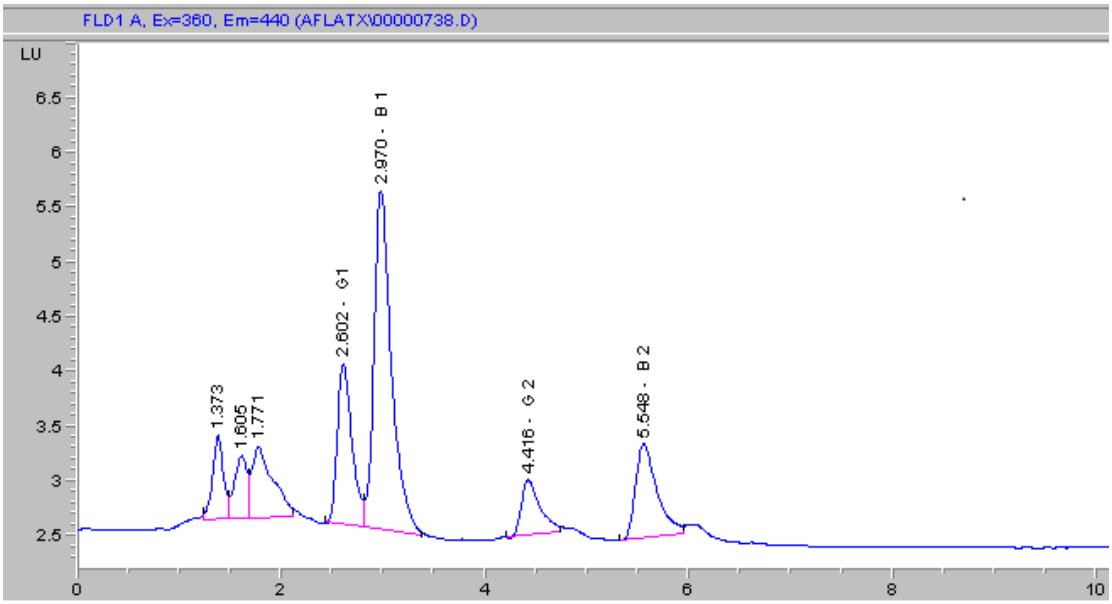

Figure (2): High Performance Liquid Chromatogram of a standard of aflatoxins (AFG_1,AFB_1, AFG_2 and AFB_2)

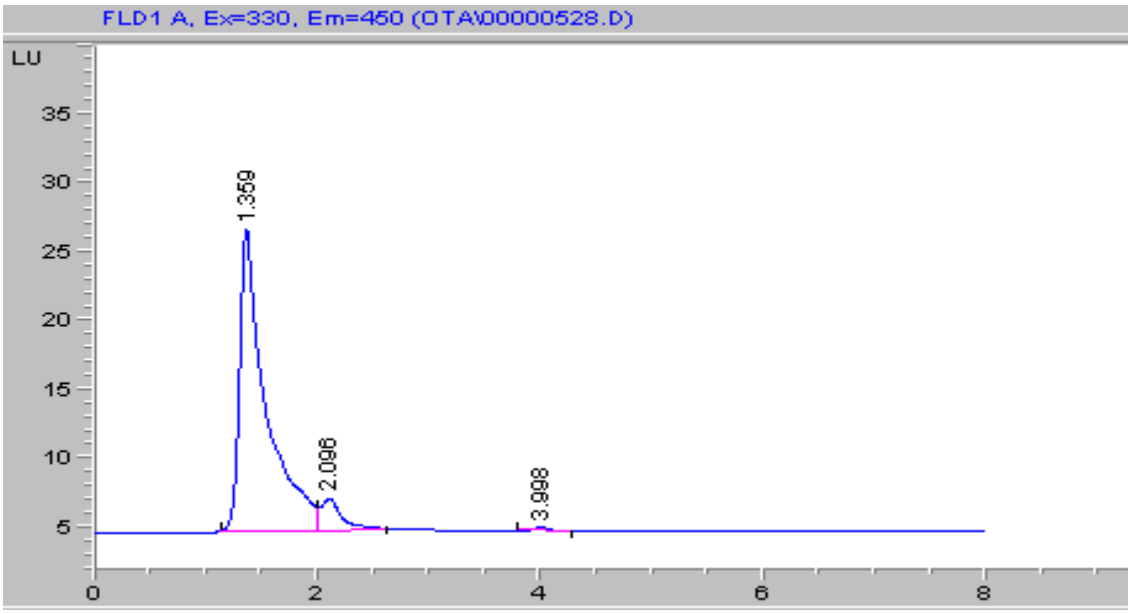

Figure (3): High Performance Liquid Chromatogram of a negative sample of aflatoxins (AFG_1,AFB_1, AFG_2 and AFB_2)

Data in Table (1) showed the percentage of infected nuts kernel and the percentage of the fungal genera which isolated in summer of 2009. Data revealed that, the percentage of fungal infected samples was 100% in all nuts samples collected from retail markets in Qassim region . The percentage averages of *Aspergillus, Pencillium* and *Rhziopus* genera infected were 57, 40, and 3.0 % in almond, 60, 32 and 8 in Pistachio, 53, 33 and 14 % in hazelnut, 50,40 and 10% in Cashew,57, 33 10% in walnuts, 67,24 and 9% in peanut collected from Qassim region , respectively. Infection of these samples with fungi may be due to the bad storage conditions in these region. Results also indicated that, fungi genera were *Aspergillus, Penicillium* and *Rhziopus* in all nut samples. It is known that, food species are common substrates for *A. flavus*, and subsequent aflatoxin production in these food stuffs is almost always due to poor drying, handling, or storage

(Arim, 1995). Jimenez *et al.*, (1991) reported moulds and mycotoxins in almonds, peanuts, hazelnut and pistachio nuts and detected aflatoxins at up to 95 mg/kg in the tested samples. The predominant fungi present in the samples were *A. flavus, A. niger, A. glaucus* Link ex Grey and *Penicillium* spp. In this concern, Vaamonde *et al*., (2003) reported that, the incidence of aflatoxigenic *A. flavus* strains was higher in peanuts (69%) than in wheat (13%) or soybeans (5%) while the ratio of CPA producers *A. flavus* isolated from all substrates was very high (94% in peanuts, 93% in wheat and 73% in soybeans). Isolates of *A. flavus* able to produce simultaneously aflatoxins type B and CPA were detected in all substrates, suggesting the possibility of co-occurrence of these toxins. While, Mphande *et al*., (2004) found that, the account of *Aspergillus* 41% in all the isolates and 98% in peanut samples. the account of *Aspergillus spp*. was 35% of all the isolates, while *Aspergillus niger* being the most prevalent (20.4%). *Aspergillus flavus / parasiticus* were also present and accounted for 8.5% of all the isolates, with *A. flavus* accounting for the majority of the *A. flavus /parasiticus* identified. Regional differences in aflatoxin contamination of crops may be attributable to climatic conditions and to agricultural practices that increase susceptibility of plants to invasion by *A. flavus* (Pildaina *et al.,* 2004).

Occurrence of mycotoxic fungi isolated from nuts :

Mycotoxins are secondary metabolites produced by specific filamentous fungi that contaminate agricultural commodities. Myctoxins produced by an active growing mold. Molds can grow actively without mycotoxin formation. Therefore, these study was carried out to isolate mycotoxic fungi namely *A. flavus* producing aflatoxins.

From Table (2) it could be indicated that, *A. flavus* was the most myctoxins- producing *Aspergillus spp* , represented, 88,78, 84, 46, 80 and 93% of *Aspergillus spp* isolates from almonds, pistachio , hazelnuts, cashews, walnuts, and peanuts collected from Qassim region, respectively were able to produce the aflatoxins.

Results of Table (2) showed that, averages the range of total af latoxins 20-40,12-60,16-50,1.2-6,30-70 and 65-150 mg /L of the yeast extract sucrose (YES) medium were produced by *A. flavus* isolates almonds, pistachio , hazelnuts, cashew, walnuts and peanuts samples collected from Qassim region , respectively. It is worthy to mention that, the current investigation to detect the aflatoxins (B_1, B_2 G_1 and G_2) levels and /or frequencies in the nut. Furthermore, Mphande *et al*., (2004) investigated 32 isolatesof *A. flavus* for their ability of mycotoxin production. They found that, 11isolates did not produce detectable aflatoxins, 8 isolates produced only aflatoxins B_1 and B_2, and 13 produced all four aflatoxins (B_1, B_2, G_1 and G_2) in varying amounts. When the raw peanut samples (n = 120) were analyzed for total aflatoxins, 78% contained aflatoxins at concentrations ranging from 12 to 329 µg/kg. A review of monitoring studies on the occurrence of aflatoxins in food products has demonstrated that aflatoxins are still being found frequently in food products at levels that are of significant concern for consumer protection (Scott and Lawrence, 1997 and Stroka and Anklam, 2002). The occurrence of aflatoxins in dried fruits and nuts was surveyed in the study by Luttfullah and Hussain, (2011) in Pakstan. They found the percentage of contamination for total aflatoxins in the samples such as in; dried apricot (20%), dates (10%), dried figs (50%), dried mulberries (26%) and raisins (20%), while in apricot kernels (26%), almonds without shell (30%), walnuts with shell (40%), walnuts without shell (70%), peanut with shell (40%),peanuts without shell (50%), pistachios with shell (20%), pistachios without shell (50%) and pine nuts with shell (20%).The highest contamination levels of aflatoxins were found in one peanut sample (14.5 mg/kg) and one pistachio sample (14 mg/kg). Molds of the genus *Aspergillus* frequently decay the kernel of pistachio nuts (Mojtahedi *et al.,* 1979). On the other hand, pistachio nuts are among the commodities with the highest risk of AF contamination (Pittet, 1998).

The obtained results of Table (3) and Figures (1,2 and 3) indicated that 80, 80, 60, 40, 40, 20 % for pistachio, peanuts, walnuts, almonds, hazelnuts and cashews samples collected from Qassim region , respectively were contaminated with aflatoxins B_1 and B_2 , aflatoxins G_1 and G_2 .The highly percentage of aflatoxins was found in both Pistachio and peanuts 80% with a concentrations of aflatoxins (AFB_1, AFB_2, AFG_1 and AF G_2) ranged from (11-90, 2-70, 1.1-55 and 1.9-25 "µg/kg") and (70-140,18-71,2.2-10.1and 3.1-13.4 " µg/kg" respectively). Figures 1, 2 and 3 shows the HPLC chromatogram of aflatoxins (G_1,B_1,G_2 and B_2) separation of standards and both positive and negative nuts sample , respectively.

Mycotoxin contamination in some edible dry fruits and nuts has been reported by Zohri and Abdel-Gawad (1993), Abdel-Hafez and Saber, (1993) and Singh *et al*., (2001). Aflatoxins were detected in 90% of hazelnut samples (25–175 mg/kg) and 75% of walnut samples (15–25 mg/kg).In a survey of peanut products in North America, 19% of 1416 samples examined were contaminated with an average level of 1 µg/kg (Stoloff 1977),In Thailand 49% of 216 samples contained AFB1 at an average level of 424 µg/kg (Shank et al. 1972).Aflatoxins are present in food chain consumption of aflatoxin in many parts of the world varies from 0 to 30 000 ng/kg/day (Denning, 1987).Some factors such as high temperature and low moisture can result in cracks in the seed and subsequent invasion by the fungus. Temperature and moisture are the dominant factors that affect aflatoxin contamination of corn. Environmental conditions most favorable for maximum growth and aflatoxin production by *A. flavus* are temperatures greater than 30ºC, maximum relative humidity of greater than 85%, and water activity of 0.98 to 0.99 (Payne *et al.,* 1988). Thus, *A flavus* can infect with proper moisture/temperature conditions during storage almost any stored product (Payne, 1992). Aflatoxin formation in groundnut is favored by prolonged end of season drought and associated elevated temperature (Rachaputi, *et al*., 2002). Singh and

Shukla(2008) investigated fungal infection and mycotoxin contamination in fresh and stored kernels of walnuts collected from different localities of Uttaranchal (India) . They found the species of *Alternaria*, *Aspergillus* and *Penicillium* were predominant. Also, they found that, Thirty-nine percent of *Aspergillus flavus* isolates were toxigenic and produced up to 2170 mg/l of aflatoxin B_1 in the liquid media. Aflatoxin B_1 was the most common mycotoxin encountered as a natural contaminant in the stored samples.

The current investigation was carried out to detect and determine the aflatoxins quantitatively (AFB_1, AFB_2, AFG_1 and AFG_2) and contamination levels in the nuts samples collected from retails markets in Qassim , region .

Conclusion

Studies were carried out to investigate the mycoflora profile and aflatoxin contamination in red nut kernels (almonds, peanuts, hazelnuts, pistachio nuts, walnut and cashew). *Aspergillus flavus, A. niger* and *Penicillium sp* the predominant fungi. The concentration of aflatoxin B_1 was high in peanut and walnut kernels as well as in the toxigenic strains of *A. flavus* isolated from these kernels. There is an urgent need to check the use of contaminated kernels and their products for edible purposes, aflatoxins are potent carcinogens which pose the risk of mycotoxicoses for the consumers.

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Locating Input/ Output point in Facility Design

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Abstract: One of important decision in facility design is determination of Input and Output points location. Input and output points location problem is an NP-Hard combinatorial problem with many applications. We consider location of input and output points on perimeter of shortest single loop path. Parameter setting is one of the most important issues of research in Genetic Algorithms (GAs). An efficient experimental design method for parameter optimization in a genetic algorithm was carried out using the Taguchi method. Genetic parameters including the population size, the crossover rate, the mutation rate, gene mutation rate and the stopping condition are considered as design factors. We investigate effect of number of AGV vehicles and their capacity on total time of AGV systems in the uncertain environment. Using simulation based optimization, we determine a robust solution and numerical results show efficiency of our solutions comparing with the result of deterministic approach.
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Keywords: Input/Output points location; Genetic algorithm; Queuing theory; Simulation optimization; Taguchi method.

1. Introduction

One of the oldest activities done by industrial engineers is facilities planning. The term facilities planning can be divided into two parts: facility location and facility layout. The latter is one of the foremost problems of modern manufacturing systems and has three sections: layout design, material handling system design and facility system design (Tompkins et al., 2003). Determining the most efficient arrangement of physical departments within a facility is defined as a Facility Layout Problem (FLP). Layout problems are known to be complex and are generally NP-Hard (Garey & Johnson, 1979). To solve industrial-sized cases, meta-heuristics and heuristics were developed.

Input/Output (I/O) points location problem is one of the principal design decision in the facility layout design problem. Potential I/O points location are considered as intersections of departments or on the perimeter of flow path. In the design of material flow path, AGV is one of the most common approaches in which a driverless vehicle is used for the transportation of material between departments. Maxwell and Muckstadt (1982) was first introduced the problem of AGV flow system. One of the common types of AGV system is single loop path that AGV vehicle moves on the perimeter of single loop to transport material flows. In real problem, production time of each department is uncertain and it makes some effect in working time of AGV system. In this paper, we consider multi vehicle with multi capacity AGV system. Due to complexity of problem, we develop a genetic algorithm (GA) and to solve uncertainty issue, we use simulation based optimization and design a robust solution based on simulations' scenarios.

The most difficult and time-intensive issue in the successful implementation of genetic algorithms is to find good parameter setting, one of the most popular subjects of current research in genetic algorithms. An efficient experimental design method for parameter optimization in a genetic algorithm was carried out using the Taguchi method. Genetic parameters including the population size, the crossover rate, the mutation rate, gene mutation rate and the stopping condition are considered as design factors. Taguchi's design of experiments has been widely used instead of trial and error method to set the best parameters. This method was used by Sukthomya and Tannock (2005) to determine the optimum setting of neural network parameters in a multilayer perceptron (MLP) network trained with the back propagation algorithm. Naderi et al. (2008) employed the Taguchi method to extensively tune different parameters and operators of simulated annealing algorithm which is used in the problem of scheduling hybrid flowshops introduced by them. This method is also used to determine an optimal process parameter setting which critically influences productivity, quality, and cost of production in the plastic injection molding (PIM) industry (Chen et al., 2009). The rest of paper is has the following structure. In section 2 problem is defined, section 3 contains GA algorithm, and computational results are given in section 4, conclusions are presented in section 5.

2. Problem Definition

I/O points location problem is a type of network queuing model in which material flows enter the queuing system from a department and exit from the system from another department. There are some assumptions:

- If an AGV vehicle is fully occupied, it cannot present a service for prepared goods and materials.
- Potential I/O points location are in intersection of departments on the perimeter of single loop path.
  Each vehicle can transport different material flows at the same time.

3. Genetic Algorithm

Genetic algorithms are based on biological evolution. They have been applied to many fields of optimization, and they have shown to be highly effective. Genetic algorithm is a population-based search approach. GA parameters are defined as follows.

- pop_size: initial population
- max_generation: maximum generations until stop
- gene_mut_rate: probability of mutation for each gene
- mutation_rate: probability of mutation for each chromosome
- cross_rate: probability of crossover for each pair of chromosomes

We use chromosome representation of Arapoglu et al. (2001) in which each gen shows a potential location selected for I/O points of department. We use two-point crossover for crossing each two children. For mutation operator, we change location of I/O point for a randomly selected department. The advantage of this representation is that there is not any clumsy after crossover and mutation. For each chromosome, we search its neighborhoods to find better solution than the case where a gen of chromosome is changed at any times. If one of chromosome neighborhood has a better solution than it, chromosome's neighborhood is considered as chromosome and this procedure continues until we have improvement in objective function. For this selection procedure, we use a tournament selection in which the chromosome with the best objective function between four randomly selected chromosomes is selected as child.

4. Taguchi method

The most exhausting issue in the successful implementation of genetic algorithms is to find good parameter setting. In this paper, we present an efficient experimental design method for parameter optimization in a genetic algorithm using Taguchi method. In an experimental design when the number of factors increases, the number of treatment combinations increases more rapidly. In these cases we can consider and examine only some of treatment combinations instead of all of them calling fractional factorial experiment. One of the approaches to deal with such experiments is Taguchi method. Dr. Taguchi introduces different orthogonal arrays for different kinds of experimental designs. Taguchi considers two types of factors in every process. First controls factors which is assigned to the inner array and directly decides the desired value of the output and second controls noise factors which are assigned to the outer array and can be measured by an appropriate signal-to-noise ratio which is measured as follows:

$$\text{S/N ratio} = -10\log_{10}^{(objective\ function)^2}$$

Taguchi classifies objective functions into three categories: the-smaller-the-better (SB) the-larger-the-better (LB), and a-specific-target-best (TB) cases. Taguchi method aims to determine best levels of control factors. In turn, the best levels of control factors are those which maximize the signal-to-noise ratios. Such a parameter design is called a robust design. In this paper there are five control factors of interest that influence the efficiency of genetic algorithm:

1. Crossover rate with four levels
2. Mutation rate with four levels
3. Gene mutation rate with four levels
4. Stopping condition with three levels
5. Population size with three levels

These control factors and levels of each are shown in Table 1. As mentioned before, since examining all treatment combinations of these factors is difficult and time-intensive, we employed Taguchi method to perform the experiment.

Table 1: Levels of each factor

Factor	Symbol	Level			
		1	2	3	4
Crossover rate	A	0.2	0.4	0.6	0.8
Mutation rate	B	0.1	0.3	0.5	0.7
Gene mutation rate	C	0.2	0.4	0.6	0.8
Stopping condition	D	10	15	20	
Population size	E	20	20	50	

Table 2. Modified orthogonal array L18

	Control factors and levels				
Trial	A	B	C	D	E
1	A(1)	B(1)	C(1)	D(1)	E(1)
2	A(1)	B(2)	C(2)	D(2)	E(2)
3	A(1)	B(3)	C(3)	D(3)	E(3)
4	A(1)	B(4)	C(4)	D(1)	E(1)
5	A(2)	B(1)	C(2)	D(3)	E(3)
6	A(2)	B(2)	C(1)	D(1)	E(1)
7	A(2)	B(3)	C(4)	D(1)	E(1)
8	A(2)	B(4)	C(3)	D(2)	E(2)
9	A(3)	B(1)	C(3)	D(1)	E(1)
10	A(3)	B(2)	C(4)	D(3)	E(3)
11	A(3)	B(3)	C(1)	D(2)	E(2)
12	A(3)	B(4)	C(2)	D(1)	E(1)
13	A(4)	B(1)	C(4)	D(2)	E(2)
14	A(4)	B(2)	C(3)	D(1)	E(1)
15	A(4)	B(3)	C(2)	D(1)	E(1)
16	A(4)	B(4)	C(1)	D(3)	E(3)

Table 3. ANOVA chart

Factor	DF	SS	MS	F	Percent	Cumulative	P_Value
A	3	6.65	2.22	1.25	4.15	20.99	0.07
B	3	7.98	2.66	1.50	8.25	46.51	0.06
C	3	2.03	0.68	0.38	10.08	51.71	0.21
D	2	10.08	5.04	2.85	20.15	84.99	0.03
E	2	2.18	1.09	0.62	4.16	91.29	0.13
Error	2	0.34	1.77				
Total	15	29.27					

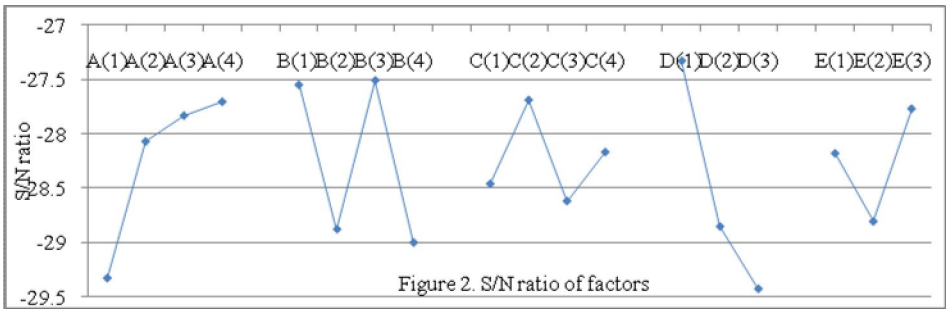

Figure 2. S/N ratio of factors

Table 4. Improvement in objective function compared with single vehicle

Size	No	Improvement%	Size	No	Improvement%
	1	15.7		26	10.1
	2	6.5		27	11.7
10	3	8.6	60	28	8.8
	4	15.4		29	10
	5	11.3		30	12.1
	6	6.5		31	7.4
	7	12		32	11.4
20	8	13.2	70	33	7.6
	9	14		34	8.8
	10	6		35	9.7
	11	13.4		36	8.2
	12	14.7		37	13.7
30	13	14.3	80	38	15.3
	14	6.8		39	8
	15	14.1		40	10.1
	16	13.1		41	14.5
	17	6.8		42	6.2
40	18	12.1	90	43	11.1
	19	15.9		44	6.9
	20	10.3		45	14.8
	21	8.7		46	7.7
	22	15.4		47	13.9
50	23	11.1	100	48	12.5
	24	15.5		49	13.1
	25	7.8		50	6.7

Total degree of freedom of these five factors is 16. Therefore, the selected orthogonal array should have a minimum of 13 rows and 5 columns to accommodate all of these factors. From the standard table of orthogonal arrays the fittest orthogonal array, L16 is selected. L16 is composed of 5 factors with 4 levels each and our problem consists of three four-level factors and two three-level factors. Therefore, we should adapt the selected orthogonal array to our experimental design. To do so, we added two extra levels to factors. We doubled level 1 of factor D and level 1 of factor E and considered them as level 4.The modified orthogonal array L18 is presented in Table 2. By implementing GA in MATLAB 7.5.0 running on a personal computer, we replicated each trial 40 times specifying 40 samples of each trial. The value of objective function gained from each run was recorded. In next step analysis of variance (ANOVA) was conducted and the relative significance of individual factors in terms of their main effects on the objective function was explored. S/N ratio of each factor is indicated in Figure 2. Table 3 shows ANOVA chart in which significant and insignificant levels are distinguished.

5. Computational results

The genetic algorithm is coded in MATLAB 7.5.0. All the test problems are solved on a Pentium 4 computer with 1024 MB of RAM and 2.26 GHz Core2 Due CPU. We solve test problems generated by Fatemi and Ardestani (2011). We implement a simulation optimization as follows. We run GA algorithm for randomly generated production time and we check which locations are selected as I/O point locations with respect to others in all scenarios and consider them as I/O point locations. In Table 4, multi capacity multi vehicle AGV system is compared with single capacity single vehicle AGV system and in Table 5, a comparison between simulation approach and deterministic approach in a situation with random production times are made.

6. Conclusions

In this paper, a GA algorithm is developed to solve I/O points location problem in multi capacity multi vehicle AGV system in uncertain environment. We show effect of AGV number and their capacity type on working time of AGV system, moreover we show efficiency of simulation optimization approach compared with deterministic approach.

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3/5/2011

Application of Cost-Benefit Analysis in Assessment of Railroad Projects; a Case Study: Gorgan- Mashhad Railroad (Iran)

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Abstract: The history of growth and development in developed countries indicate that transportation has an important role in economic growth of these countries, such that most scientists and specialists of economic affairs believe that the transportation industry is the axis of growth and development in Europe and America. In Iran, also, transportation has been introduced as the key to development, and among the different routes of transportation, railroad has an important role in this respect. Considering the large volume of required investment for railroad projects, it is evident that scientific studies are needed to evaluate whether or not these project are economical. In this study, we have used the method of cost-benefit analysis and concluded that establishment of Gorgan-Mashhad railroad is economical.

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Keywords: Cost-Benefit Analysis; transportation; economic; development

1. Introduction

Having access to markets is an important point in the capability of a region to have a successful performance in the integrated network of world trade. Owing to the role they play in the facilitation of access to the site of companies, enterprises, and also households, transportation vehicles and systems are so important. Transportation is considered as the most important determinant factor in industrial locating. In any economic system, either advanced or primitive, there are some degrees of necessity for transportation of materials, goods, man, ideas, or innovations, since all economic activities of man are not gathered in one place. Investment in transportation can have long-term and extensive environmental and socioeconomic effects. Therefore, the decisions related to policy making and programming for transportation mostly lead to outstanding effects on economic development, through influencing on consumable costs and the state costs, employment opportunities, consuming the resources, efficiency, quality of local environment, price of real estates, the economic potentials, and wealth accumulation.

Advancement and development of countries is accompanied with an increase in the volume, inputs, and outputs of the economic system, which relies on the development of transportation system. Developed industrial countries depend extensively on remote raw materials to maintain the flow of production on the one hand, and on the other hand having the greatest effort to achieve global markets.

Thus, the international transportation of goods is so extensive. From the structural point of view, transportation network is so complex, such that transportation is an objective necessity for global economy as well as political powers. Transportation is defined as carrying goods, passengers, or ideas from one place to another place. After the end of World War II, most governments and local authorities tried to improve their transportation communications with other regions. The main communication route among the regions is railroad and the regions that have such communication routes enjoy a higher level of regional development compared with other areas. Thus, most regions try to establish this route to improve their communication with other centers and also benefit from its economic effects.

The inter-city routes and highways between different regions are the next important route of communication between different areas that facilitate the access of different regions. Technological advancements and transportation of goods by tracks have led to flexibility in the flow of goods distribution and several new centers for economic activities have been created in small towns.

Moreover, air transit has developed new communication. However, because of its specific properties, railroads have maintained its position as a powerful route of transportation.

It is stated that railroad industry requires great investments and the industry observes specific regulations and it has a bad reputation in resistance against changes. However, in comparison with other transportation systems, railroad transportations have many advantages. For instance, we can point to some of its advantages such as safety, travel comfort, low

costs, fuel consumption providence, lower air pollution, high capacity in carrying passengers and goods, and less land occupation.

Establishment of Gorgan-Mashhad railroad is one of the state projects to expand the railroad system of the country, which as an essential infrastructure can improve the socioeconomic state of the northern part of the country.

Considering the positions of Mashhad (as the second metropolitan area of the country), and Bojnurd and Gorgan (as the capital cities of the northern Khorasan and Golestan provinces), along with some other important cities such as Ghuchan, Shirvan, and Gonbad, establishment of a railroad network in the northern part of the country seems to be necessary.

Moreover, establishment of this network would make the communication of towns in the northern parts of these two provinces with other provinces possible. In future, establishment of Sari-Bandar-e Anzali railroad would provide a safe railroad route between the northeastern and northwestern parts of the country. Moreover, this railroad would communicate the towns in north part of the northern Khorasan province and its capital, Bojnurd, to Tehran. Regarding the direction of the route along the boundary of Iran-Turkmenistan, this route can serve as a communication road among the towns in the northern parts of these two provinces and Turkmenistan and be considered as a route parallel with east-west corridor.

2. Methodology

Cost-benefit analysis is one of the most important analyses of economic assessment.

The history of cost-benefit analysis (CBA) shows how its theoretical origins date back to issues in infrastructure appraisal in France in the 19th century. The method quantifies all costs and main benefits of the project. The major strength of cost-benefit analysis is that the method considers the costs and benefits of different alternatives of the project in a relevant framework. Therefore, the results are presented as a range of the choices that are converted into comparable values.

The cost-benefit analysis provides a protocol (a set of defined concepts) for estimation of the effects of suggested policies on the effectiveness, which can help us determine whether or not the expected results (outcomes) are beneficial for people. This analysis has become a common method in evaluation of public projects; for instance, construction of a new urban highway, construction of a new airport in site A or B, making decision on issuing a certificate for further excavation of minerals, or environmental assets. These are the decision for which cost-benefit analysis can be employed. In fact, one of the interesting aspects of cost-benefit analysis is its applicability in many different areas.

Besides, cost-benefit analysis is a fundamental and technical theory in neoclassical wealth economy, which is consistent with the patterns of neoclassical sub-economy and emphasizes on the role of prices in assigning the resources, and is widely used in economic evaluation of projects.

According to the United Nations Organization, cost-benefit analysis is defined as a method for evaluation of desirability of a project through comparison of the incomes of the project with its costs, where the prices are transformed into current values with an appropriate official decrease.

Cost-benefit analysis is providing a practical solution for determination of desirability of the projects from the viewpoint of their effects during the time and also their range of action in different industries and regions. To achieve this, the costs and benefits should be calculated and determined.

This is the method of identifying, describing, and evaluating the factors that should be addressed in a rational and economic decision, and in fact, it requires some modifications in the concepts of current cost and benefit, which reflects the objectives, bases, and social constraints instead of individual constraints, in evaluation of investment projects.

However, it should not be ignored that cost-benefit analysis as a method for information flow is progressive. Thus, in spite of the problems that are encountered because of scarcity of required statistics or because they need predicting the application, this analysis is the most useful framework for evaluation of projects and programs related to development of resources.

One of the advantages of employing cost-benefit analysis is the help of this method in removing the need for centralization of decision making centers in the economy. Even if the public sector is small, no central government would be able to make decisions on the number and types of required public projects just by relying on the current information. The presence of a decision making center for calculation of costs and benefits of each investment project is essential. Therefore, to facilitate the improvement of cost and benefit determination of a project, decentralization of decision making centers is necessary.

Accordingly, cost-benefit analysis is a practical method for assessment of the profitability of projects. For better fulfillment of the method, the current and future conditions of the community should be carefully evaluated. Many projects that are

not currently useful for the community may be so advantageous in future.

In general, it should be mentioned that the need for cost-benefit analysis roots in the viewpoint that the following items should be observed in any investment in public sector:

a) Considering the complete set of social costs and benefits resulted from a project in the national economy,

b) Valuation of inputs with respect to the costs of their final social opportunity and also outputs with respect to their final social value,

c) The future decrease in costs and benefits which were correctly evaluated according to the social reduction rate.

Cost-benefit analysis is not a theory by itself; rather it is technique with the aim of providing a set of appropriate and systematic theoretical regulations and methods for directing public sector investments. It is usually fulfilled to help establishment of policies in a specific framework. According to R. Sagon and A. Williams, employment of cost-benefit analysis provides a framework for public decision making, which will lead to more responsible decision making.

A private investor does not mostly evaluate the non-economic and social aspects of his desired project. For a private investor, if he knows his goals, it would be easier for him to select one project among different ones and all he needs to know is that which project satisfies his goals in the best way.

However, in social cost-benefit analysis, income of the project is not the true index for social costs, and since social costs and benefits are different from the individual ones, investment decisions on the basis of commercial benefits may lead to a series of wrong decisions with respect to social wealth, which should be observed by the government.

The main guideline for cost-benefit analysis can be summarized in the following five stages:

1) Determining all the outcomes related to a specific decision that is linked with public policies,

2) Estimation of the monetary value of all these outcomes, such that the value of cost flow and assignable social benefits can be obtained,

3) Estimation of the current pure value of these costs and benefits using a decrease rate,

4) Comparison of the current pure value of costs with the current value of benefits in a general ratio of cost-benefit,

5) Selection of the items in which the cost-benefit ratio is smaller than one and then selection of the choice with the least ratio of cost-benefit.

Studies on estimation of load and passenger demand

- Evaluation of load demand:

Carrying goods is one of the main reasons for establishment of railroads. In fact, railroad is the safest and most economic route of carrying goods in large distances.

The main exported goods of Khorasan province are brimstone, urea, and میلگرد, and the main imported goods are corn, ironware, and gasoline. The weight of exported and imported goods of the province were 6978 and 6888 thousands of tons, respectively, and in the same year, 8593 thousands of tons of goods were transported in the province.

The main exports of Golestan province are wheat, potato, and chipboard, and the main imports are cement, gypsum, and corn. The amount of imported and exported goods were 2473 and 2519 thousands of tons, respectively, and 678 tons of goods were transported in the province.

Transit load demand:

Most specialists believe that Iran has a great potential for transit of goods, which is be considered as export. Some countries such as Turkey, Bulgaria, Germany, and Netherlands that are in the route of goods transit have special advantage and thus earn a large amount of income through transit of goods.

Similar to the above-mentioned countries, Iran is located in the route of goods transit and also in the route of Baltic, Caucasian, middle Asian, and Slav countries, and thus has an outstanding position in this respect. Thus, the country can have a better performance in this respect.

In the transit sector, most transported goods in the descending order were fuel materials (730,000 tons, 17%), cotton (523,000 tons, 13%), foods (273000 tons, 7%), constructional materials (236,000 tons, 6%), and vegetable oils (203,000 tons, 5%). Moreover, the main origin of most transits was the United Arab Emirates (40%), and the main destination of most loaded transits was Afghanistan (22%). The most active boundaries in imported transits were Bandar Abbas (1,744,000 tons, 42%), Serakhs (1,140,000 tons, 27%), and Bazargan (569,000 tons, 14%).

It should be noted that besides the 4,183,590 tons of transited goods in the country, 3,885,041 tons of oil materials were imported in the SWAP framework in Neka and Noushahr ports. Thus, the 12-month statistic of the total transit in the year 2003 was 8,068,631 tons, which was increased 68.81%, compared with the time period of the past year.

Of the 4,183,590 tons of goods transited in Iran, the share of railroad and road were 28% and 72%, respectively.

During the 12 months of 2003, 730,000 tons of oil materials (17%) and 3,454,000 tons of non-oil materials were transited through Iran.

Concerning the transit load demand for Gorgan-Mashhad axis, we can consider the goods from the origin of Turkey, Iraq, and Azerbaijan to Turkmenistan, and vice versa as the potential transit of the route. In this respect, we could have 236,337 tons of transited goods in this axis.

- Evaluation of passenger demand:

Carrying passengers is one of the main goals of railroad establishment. Here, by considering the statistics of road passengers and calculating an appropriate absorption rate, we would predict the number of railroad passengers.

Costs of the project

- Costs of fulfillment of the project:

The costs of fulfillment of the project are the costs from the initiation of the preliminary studies to the end of establishment and operation of the railroad. Thus, the costs can be classified as follows:

- The costs for preparation of executive maps and application of the route,
- The costs for buying the lands and removing the obstacles in the surrounding of the rout,
- The costs of executive steps of the project,
- The costs of providing and installing the signs,
- The fees of consultant supervising engineers, senior supervisors, laboratory, research, and qualitative controls, and
- The costs of transportation vehicles.

The costs of fulfillment of the project were presented in table 1.

Table 1- Summary of the establishment costs of Gorgan-Mashhad railroad

Section		Cost (10^9 Rials)
Engineering searches		27.5
Surveillance, higher surveillance, experiments and qualitative controls		178.5
To own estates		105
Run	Bed construction	1209
	Upper construction	1212
	Bridge	385
	Tunnel	215
	Stations	335
	guidelines	457
Buying fleets		802
Total		4926

The prediction of operation and maintenance costs of the axis, including the different details of the costs during the project in different years and with different methods of repair and maintenance, and putting the project into operation are provided in the annexed table.

a) Costs of repair and maintenance of the railroad:

Repair and maintenance operation consists of the three major sections of current maintenance, improvement, and repair of the route.

b) Costs of repair and maintenance of the fleet:

The costs consist of the annual costs of repair and maintenance of locomotives and load wagons.

c) Costs of operation:

The costs of operation consist of:

1: human force/ operation force costs, and

2: costs of fleet fuel and oil

The income of the project

a) Benefits from fuel consumption providence (diesel and oil).

Table 2- K and n values for UIC formula

	Bed construction - technical buildings - buildings	Upper construction	Guidelines and communications	Fleet
K	0%	7%	1%	3%
n	50	35	35	30

Through changing people's behavior, railroad transportation will lead to less frequent use of automobiles and thus improved environmental condition. Providence in fuel consumption is not only an advantage of the project, but also one of the necessities of fulfillment of the project; since by a decrease in the amount of non-renewable resources and the growth in their consumption, a serious providence in oil products and searching for practical methods in this respect are inevitable.

As transportation sector is the largest consumer of fuels, replacement of road transportation with railroad transportation will be an efficient method in fuel providence. Employing this approach, a significant providence in fuel consumption will be achieved and people will enjoy its advantages.

With regard to the amount of providence, various studies have been carried out. According to the director of Iranian Environmental Protection Agency, the fuel consumption in road transportation in Iran is 9.3 folds of that of the railroad transportation.

In 2003, the means of transportation in Iran were as follows: 116 million passengers by bus and 9.99 million passengers by minibus and cars. Thus, 52%, 44%, and 4% of passengers were carried by buses, minibuses, and cars, respectively.

Now, considering 15, 18, and 60 ml of fuel consumption for each person-km with bus, minibus, and car, if the passengers who travelled by province or local railroads, travel by road vehicles, then fuel consumption for buses, minibuses, and cars in 2012 will be 9226, 3475, and 1053 m3, respectively, and in total it will be 13755 m3. This is while the consumed fuel of passenger locomotives will be 5119 m3, and thus 8636 m3 of fuel providence will be achieved. The mentioned providence will be 7583 and 1053 m3 in diesel and gasoline, respectively.

- Fuel providence in load fleet:

The diesel consumption in trucks is 25 ml per km-tons of loads. Therefore, in 2012, 42437 m3 of diesel will be consumed. This is while the consumed fuel of the railroad fleet will be 12566 m3, which shows 1053 m3 fuel providence.

- Total fuel providence:

The total consumed fuel in road transportation will be 55138 m3 of diesel and 1053 m3 of gasoline, which is in total 56191 m3.

The total consumed fuel regarding the main, maneuver, and standby locomotives will be 20288 m3. Thus, 34850, 1053, and 35903 m3 of diesel, gasoline, and total fuel providence will be achieved, respectively.

- Value of fuel providence:

Considering the price of each liter of gasoline and diesel to be 2700 rials (international fuel prices), the value of fuel providence will be 89 billion rials. This can be regarded as one of the benefits of the projects.

- Oil providence:

In general, oil consumption in railroad transportation is 1.1% of the fuel consumption. But in road transportation, the oil consumption is about 2% of the fuel consumption. Therefore, using railroad transportation, 0.9% providence in fuel consumption multiplied by the fuel providence coefficient in railroad will be achieved. The road fleet for transit of goods and passengers that is replaced by railroad transportation in 2012 would use 1124 m3 of oil, and considering the 355 m3 of the consumed oil by the railroad fleet, 769 m3 of oil providence will be achieved.

- Value of oil providence:

Considering the price of each liter of oil to be 16500 rials, the value of oil providence will be 13 billion rials.

- Total value of fuel and oil providence:

The total value of fuel and oil providence achieved by replacement of road transportation by railroad in 2011 will be 100 billion rials.

The total value of fuel and oil providence in the coming 20 years is estimated to be 4167 billion rials.

b) Ticket sales or transit fare:

The fare of railroad that is obtained from the passengers and owners of goods would be obtained by road drivers, if railroad is not established. Thus, the income of railroad is achieved at the cost of a decrease in income of other sectors. In other words, the fare obtained by railroad sector is not social benefit; rather it is a replacement of incomes.

Hence, in calculating the social benefits of the projects for public projects, we should only consider the benefits that were not achieved with any other sectors before the implementation of the project and they would be achieved through application of the project and not only the change of combination of benefits and incomes that are currently present in the society. To this end, we have removed the railroad fares in evaluation of the social benefits of the project.

According to the specified fees, the obtained income by transportation of goods and passengers will be as follows:

Passengers:

The total route in the coming 20 years: 890 billion rials,

Goods:

Import and export in the coming 20 years: 1479 billion rials,

Transit in the coming 20 years: 777 billion rials,

Local transportation in the coming 20 years: 3122 billion rials, and

Loading in the total route in the coming 20 years: 5377 billion Rials

c) Decreased road accidents:

Each year, road accidents cause great losses to different countries. According to the World Health Organization, more than one third of the victims of accidents in the world are from third world countries.

Almost one percent of the national gross production of our country is wasted due to ignorance of the safety of the country's traffic. According to the estimates, the cost of road accidents in Iran is almost 340 billion Toman annually. This is while, the emotional stress of injured and families of victims of road accidents and the negative effect of these stresses on their socioeconomic performance is not included in the calculations, otherwise the loss would be much higher. In most cases, trucks and other heavy vehicles directly and indirectly play a major role in occurrence of road accidents.

Establishment of the aforementioned railroad would result in a significant decrease in the number of trucks, trailers, and minibuses on the road, and consequently the number of accidents and their costs. According to the studies of the comprehensive Iranian transportation project, establishment of railroad would decrease the costs of road accidents by 40%.

According the transportation model across the country, the basis of the calculations was as follows:

- Number of passengers of a car: 3 persons,
- Number of passengers of a minibus: 17 persons
- Number of passengers of a bus: 20 persons, and
- Mean weight of a truck load; 11 tons.

In 2002, inter-city load and passenger road transportation was 83.603 billion tons-km and 51.950 billion person-km, respectively. The number of travels was 12.851 million of vehicles, and the

number of travels of loading trucks was 14.872 million. The mean distance passed by passenger transportation vehicles and loading vehicles were 222 and 485 km, respectively. Thus, in 2002, 10058 million vehicle-km were transported.

The loss of road accidents in 2002, which led to 21 thousands deaths and injuries, was almost 7800 billion rials. Thus, the loss per one million vehicle-km was almost 775 million rials.

With regard to the mean number of passengers in public vehicles as 18 persons and the mean loading weight of trucks to be 11 tons, and considering the estimated loading and passenger in Section C and the length of the route to be 585 km, in 2012, we would have 161 million vehicle-km of transportation. Then, considering the 775 million rials loss per one million vehicle-km transportation, the safety value in 2012 will be 125 billion rials.

D- Scrap remaining after the project:

At the end of projects, usually some instruments will remain that are completely worn out and they are sold as scraps. Since the life of some other instruments and facilities is longer than the general duration of the project or as they were bought during the project, at the end of the project they are still usable and can be sold as used materials or be used in similar projects. Regarding this project, it can be noted that all remaining scrap is ironware, because the major portion of locomotives and passenger wagons and all parts of the loading wagons and rails are made from iron. Considering the instruments that can still be used, at the end of the project they will be valued by the direct line depreciation method.

For pavement, superstructure, buildings, bridges, communication signs, and the fleet, the scrap value is calculated by UIC formula as follows:

$$R_m = (V - K)\frac{(1+i)^n - (1+i)^m}{(1+i)^n - 1} + k$$

where Rm is the remaining depreciation value of instruments, buildings, installations, pavements, and superstructures in the desired year m, V is the basic value, K is the recycling value as a percentage of the basic value, i is the interest rate, n is the effective life in years, and m is the year for which the depreciation value is calculated.

Considering the above-mentioned formula, the total depreciation value for the project is 3011 billion rials.

On the basis of the estimations, the project will be carried out in four years.

E) Employment opportunities brought by the project:

The project can lead to some employment opportunities during its fulfillment and operation. Since according to the standards of the Iranian Management and Programming Organization, on average 100 million rials of investment is needed for one employment position, then the value of provided employments (for 660 persons) would be 66 billion rials.

3. Results of economic evaluation of the project

The costs and incomes are provided in Tables 2 and 3.

Table 3- Goods and passenger request of Gorgan-Mashhad

Year	Internal Goods (1000 tones)	Transit goods (1000 tones)	Export & Import goods(1000 tones)	Province passenger (1000 persons)	Local passenger (1000 persons)
1390	1280	185	543	705	716
1391	1348	194	582	723	724
1392	1416	203	621	741	731
1393	1485	213	660	760	738
1394	1553	222	699	779	746
1395	1621	232	738	798	753
1396	1689	241	777	818	760
1397	1757	250	816	838	768
1398	1825	260	855	859	776
1399	1894	269	893	881	784
1400	1962	279	932	903	791
1401	2030	288	971	925	799
1402	2098	298	1010	949	807
1403	2166	307	1049	972	815
1404	2235	316	1088	997	824
1405	2303	326	1127	1022	832
1406	2371	335	1166	1047	840
1407	2439	345	1205	1073	848
1408	2507	354	1244	1100	857
1409	2575	363	1283	1128	866

- Calculation of profitability of the project (without considering the current values):

With regard to the current costs and incomes, this part of the project will lead to 2069 billion rials of economic loss from the national viewpoint, during its life. However, if we consider the inflation rate to be 14%, the profitability of the project during its life will be 21885 billion rials.

- Calculation of the internal return rate:

- The internal return rate, which in fact balances the project from the aspects of income and cost, is 9%.

Table 4- Cost and income fitting (without growth), numbers are in 10^9 Rials

Year	Investment costs	Using costs	Maintenance costs	Buying fleet costs	Costs sum	Direct incomes	Indirect costs	Profit
1386	652	0	0	0	652	0		-652
1387	988	0	0	0	988	0		-988
1388	1078	0	0	0	1078	0		-1078
1389	1434	0	0	354	1789	0		-1789
1390	0	38	22	11	70	128	116	173
1391	0	35	21	20	76	125	112	161
1392	184	34	21	10	249	122	109	-18
1393	0	33	24	10	67	119	107	159
1394	0	31	23	10	64	116	104	156
1395	49	29	22	10	109	113	101	106
1396	13	28	21	27	88	111	99	121
1397	0	27	20	9	56	108	97	149
1398	0	26	19	9	53	106	95	147
1399	95	24	17	9	145	103	93	50
1400	0	24	18	9	51	101	91	141
1401	0	23	39	11	72	99	89	116
1402	0	22	36	9	66	97	87	118
1403	0	21	33	8	62	95	85	118
1404	0	20	14	11	45	93	84	132
1405	0	19	13	8	40	91	82	133
1406	0	19	12	8	39	89	81	131
1407	44	18	11	8	81	87	80	85
1408	0	17	12	8	37	86	78	127
1409	0	17	11	0	28	84	77	133
Sum	4537	503	408	558	6007	2072	1866	-2069

Table 5- Cost and income fitting (with 14% growth), numbers are in 10^9 Rials

Year	Investment costs	Using costs	Maintenance costs	Buying fleet costs	Costs sum	Direct incomes	Indirect costs	Profit
1386	981	0	0	0	981	0		-981
1387	1703	0	0	0	1703	0		-1703
1388	2129	0	0	0	2129	0		-2129
1389	3246	0	0	801	4048	0		-4048
1390	0	97	56	28	181	331	300	450
1391	0	105	63	58	227	371	334	478
1392	628	114	71	35	848	414	373	-61
1393	0	127	95	39	260	464	416	619
1394	0	138	104	44	286	519	465	698
1395	248	150	110	49	558	580	519	542
1396	74	165	124	157	519	650	581	711
1397	0	180	136	63	378	727	651	1000
1398	0	197	144	70	411	815	729	1132
1399	836	215	153	79	1284	912	817	446
1400	0	240	185	90	514	1022	917	1425
1401	0	263	449	122	834	1146	1029	1342
1402	0	288	473	114	875	1285	1157	1567
1403	0	317	498	129	944	1442	1300	1797
1404	0	349	240	194	783	1618	1462	2297
1405	0	385	258	166	809	1817	1645	2653
1406	0	425	277	187	889	2041	1852	3004
1407	1159	470	297	212	2138	2293	2086	2241
1408	0	526	349	240	1115	2578	2350	3814
1409	0	582	375	0	957	2900	2649	4592
Sum	11006	5336	4455	2877	23674	23925	21633	21885

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Phonetic Processes in Ruini Dialect

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Abstract: The article, after introducing the consonants and vowels, discusses the impacts of them on each other as a result of collocation and subsequent changes. The research not only introduces a vanishing dialect, but also it is surely a step forward to protect the cultural heritage of the Persian language. The research method is field-library, in which the researcher has done conversations with different native speakers and residents of Ruyin village. The language corpus consists of native morphemes belonging to the dialect and those items with different pronunciation in contrast to standard Persian.
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Keywords: Ruyin; consonant; vowel; phonetic process

1. Introduction

Ruyin is located in a valley 24 km north of Esferayen-57°, 29′ longitude 37°, 12′ latitude, and 1650 m [1] average altitude .A river passes through the village. There are three quarters (Barzan in Persian): Bala Deh (the upper quarter) Miyan Deh (the middle quarter), and Payin Deh (the lower quarter). The native language is Ruyini-one of the dialects of Khorasani Persian. There has been no research available on the dialect so far, whether by the native or foreign scholars.

2. Ruyini Phonemes

A. Consonants

1. Plosives: These sounds are articulated through a closure in a closed mechanism [2].

1.1. /b/: pulmonic, exgresive, voiced, plosive, bilabial
1.2. /p/: pulmonic, exgressive, tense, voiceless, aspirated, plosive
1.3. /d/: pulmonic, exgressive, lax, voiced, plosive, oral, dental
1.4. /t/: pulmonic, exgressive, tense, voiceless, aspirated, plosive
1.5. /k/: pulmonic, exgressive, tense, voiceless, aspirated, plosive
1.6. /g/: pulmonic, exgressive, lax, voiced, plosive, palatal
1.7. /q/: pulmonic, exgressive, voiced, plosive, uvular
1.8. /?/: pulmonic, exgressive, tense, voiceless, plosive, glottal
1.9. /z/: pulmonic, exgressive, voiced, plosive, fricative
1.10. /s/: pulmonic, exgressive, voiceless, fricative
1.11. /ž/: pulmonic, exgressive, voiced, fricative
1.12. /š/: pulmonic, exgressive, tense, voiceless, fricative
1.13. /f/: pulmonic, exgressive, voiceless, fricative, labio-dental
1.14. /v/: pulmonic, exgressive, voiced, fricative, labio-dental
1.15. /x/: pulmonic, exgressive, voiceless, fricative, uvular
1.16. /h/: pulmonic, exgressive, voiceless, fricative, glottal
1.17. /č/: pulmonic, exgressive, voiceless, fricative, alveo-palatal
1.18. /r/: pulmonic, exgressive, voiced, alveolar, trill [3]
1.19. /m/: pulmonic, lax, voiced, bilabial, nasal
1.20. /n/: pulmonic, exgressive, lax, voiced, alveolar, nasal
1.21. /l/: pulmonic, exgressive, voiced, alveolar, lateral
1.22. /y/: pulmonic, exgressive, lax, voiced, glide, palatal

B. Semi-vowels

There is no stop, friction, or trill once these kinds of sounds are articulated. Thus, they are labeled as semi-vowel s (glides).The only example is [W]- a bilabial voiced sound with parallels in English and Kurdish.

C. Vowels [4]

1. /i/: front, closed, long, non-labial
2. /e/: front, half-open, half-extended, non-labial
3. /a/: front, open, open-extended, short
4. /u/: back, closed, round, long, labial
5. /o/: back, half-open, half-round, short, labial
6. /â/: back, open, round, long, labial

D. Diphthongs [5]

The phonetic structure of these sounds will be treated prior to describing each sound. /âo/ consists of two components: a vowel- /â/ and a non-syllabic vowel- /u/ which is labialized and changes into /w/.

Therefore it is distinguished from the vowel /u/ in terms of sonority. The first component is articulated in a more sonorant manner. The dialect's diphthongs are composed of two vowels inside a syllable, while they have their own characteristics. The second component is not a vowel at all, yet similar to /i/ and /u/ with no tenseness. Thus, they are called semi-vowels in a right sense. They change into /y/ and /v/ before vowels, eg, pay–pey.
/ey/ in genitive phrases is divided into two syllables, eg, pâ.ye kuh (foot of the mountain)- /y/ is accompanied by–e genitive marker [6].
/âw/ as in je.lâ.ve panjere (in front of the window)- cf. jelow (front) is seen in Persian, eg, de.ro.ve gandom (harvesting)- cf.derow kardan (to harvest).

E. Description of Diphthongs
1. /âw/: consists of two simple vowels-/â/ and /u/.
2. /âo/: consists of two simple vowels- /â/ and /o/: back, half-open, half-round.
3. /oe/: consists of two simple vowels- /o/ and /e/: half-open, short.

F. Phonetic Processes
These are changes resulted from collocation of sounds and are considered as language universals.

Ellipsis: The process includes deletion of one or more sounds in Persian morphemes used in Ruyini, as below examples:

/h/ is often elided before and after long vowels:

Ruyini	Standard Persian
izom firewood	hizom
kelo hat	kolâh
koe mountain	kuh

/h/ is elided in morphemes' middle and ultimate positions:

bes kiss	buseh
bitar better	behtar

Ellipsis takes place in a number of Persian morphemes to make the pronunciation easier:

aynah mirror	âyineh
asiyo mill	âsiyâb

3. Addition

The process takes place when one or two phonemes are added to the Persian morphemes:

gilors (/r/ is added)	gilâs	cherry
gezenda (/d/ is added)	gazane	nettle

4. Substitution
It includes inversion of a phoneme in a morpheme to other phonemes. The process is not so frequent, yet it takes place in a big number of morphemes of such a kind [7]:

The short vowel /a/ changes to /âo/ before /f/ and /b/ in middle and ultimate positions, the two consonants will be deleted subsequently:

lâo lip	lab
šâo night	šab

The long vowel /u/ changes to /i/:

zerdali apricot	zardâlu
sizân needle	suzan

The short vowel /a/ changes to /e/;

gevân goat's-thorn	gavan
šeqâm turnip	šalqam
behor spring (season)	bahâr
gendum wheat	gandom

The short vowel /e/ changes to /a/:

aynak glasses	eynak
pašnah heel	pâšneh

The long vowel /â/ changes to /o/:

bod wind	bâd
siyoh black	siyâh

The short vowel /o/ changes to /i/:

kilâng pick	kolang
rišân bright	rôšan / rowšan

The consonant /b/ changes to /v/:

tavistân summer	tâbestân

aftava âftâbe
ewer

/l/ changes to /r/:
lâr lâl (âb-e raked)
stagnant water
zelor zalâl
limpid

/r/ changes to /l/:
zenjil zanjir
chain
sebidoel sepidâr
aspen

The long vowel /u/ changes to /oe/:
toet tut
mulberry
toep tup
ball

5. Saturation

It is the opposite of reduction and takes place where short vowels of Persian morphemes turn to long vowels in Ruyini:

The short vowel /a/ changes to the long vowel /â/:
kâm kam
few/little
sang sang
stone

The short vowel /o/ changes to the long vowel /u/:
čequndar čoqondar
beet
rub rob
sauce

The short vowel /e/ changes to the long vowel /i/:
kišt kešt
plantation
atiš âteš
fire

5. Reduction

The process takes place where long vowels are reduced to the short ones:

5.1. /â/ is reduced to /a/:
ašno âšnâ/âšenâ
familiar/acquainted
astin âstin
sleeve

5.2. /i/ is reduced to /e/:
bel bil
shovel
bex bix
bottom/ lower part

Coalescence

In a number of cases, two morphemes are combined to form a double-letter in spelling (harf-e tašdid dâr/ mošaddad):
putta bute
bush
gulla golule
bullet

Inversion

The process involves replacement of one or more phonemes in Persian morphemes:
sebând espand
qelber qarbâl
riddle
qulf qofl
lock

Fracture

The morphemes are broken down into smaller units. The process is confined to the proper nouns in Persian:
madasan mohammad hasan
two male names
madeseyn mohammad
hoseyn two male names
mandali mohammad ali
two male names

Different people including Armenians, Uyghurs, Ardabilis (from Ardabil, in Azarbayejan), and Kormanji kurds have immigrated to Ruyin in past times, each people with their own impact on Ruyini sound system:

a. The vowels /â/ and /e/ with a high frequency are originated from Armenian:
Ruyini Persian
nâng nang
disgrace
ez az
from/ of/ since

b. /âo/ is a high frequency diphthong and a historical remainder of The Old Iranian /au/ and Avestan /ao/. [8].

c. /oe/ is a remainder of the Old Iranian /ayū/ which turns to Parthian /ē/. Therefore, it must have developed to Ruyini /ē/, or /e/. It should be noted that Ruyin is located in ancient Parthian, alternatively, the sound could have been borrowed form Uyghur, or Ardabili Turkish.

d. /âw/ is either the Old Iranian final -ava, /aw/ in Parthian and /âw/ in Ruyini, or The Old Iranian âvaya, Parthian /âw/ and Ruyini /âw/. [9].

e. The direct impact of Kormanji Kurdish can be seen as /w/ in a number of Ruyini morphemes.

f. The classification of phonetic processes is not definite one, thus overlapping is probable in a number of cases [10]. Language is a phenomenon, so the dialect has been studied with regard to its geographical situation, the counter influences of the adjacent dialects, and different social, political and cultural factors, all resulted in development of a certain dialect-Ruyini.

g. Ruyini people call themselves as Revini. It is suggested that the word is made up of two morphemes: rev+ in.

g.1. The former morpheme is developed from the Middle Persian–ēn which is a developed form of the older morpheme –aēna, a relativity suffix [11].

g.2. –in is a developed form of the older–âyna which is a suffix of place.

Prof. Garnik Asaturian suggests that Revin is developed from Râvâyna meaning the residence of Râvs, one of the Iranian tribes in ancient times. Ruyini people identify themselves as Iranians and have kept close ties with other Iranian people, although they are linguistically a distinct identity.

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The jurisdiction and discretion challenge of International Court of Justice in Kosovo opinion

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Abstract: The advisory opinion of the international court of justice (here after ICJ or the court) is a legal constitute which comes from common law system. It is established to aid other bodies of united nation and specialized organizations in their activities according to their statutes. This paper is about to analyses the reasoning of the court on establishment of its jurisdiction and discretion in Kosovo opinion. In this respect, it will consider the question raised by General Assembly or (GA), the articles of charter and statute of the court in this issue, the reasoning of the court and declarations of the judges in favor or against the court reasoning, and the effect of this opinion on development of international law. It seems, this is the first time that the court does not deal with the conduct, rights and obligations of states or international organizations, but rather deals with an action of non- state entities which the legality of their action on issuing the declaration of independence according to international law must be determined.

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Keywords: International Court of Justice; jurisdiction; discretion; Kosovo opinion; advisory opinion

Factual background

Kosovo declaration of independence was issued on February 17, 2008[1], after ten years of international community efforts for solving the conflict whit Serbia.

The first action in this respect was about concluding Rambouillet accords which provided, inter alia, for a three years interim democratic self-government in Kosovo, albeit within the Federal Republic of Yugoslavia (FRY). The accords were accepted by Kosovo (on 18 March 1999), but rejected by Serbia. After NATO's military intervention, the security council (SC) adopted resolution 1244 (1999) authorizing interim international territorial administration of Kosovo, the creation of United Nations Mission in Kosovo (UNMIK), and implementation of provisional institutions of domestic self-government [2].

Local political institutions were established in 2001 on basis of regular democratic elections. Gradually, powers and responsibilities were transferred from UNMIK to Kosovo authorities. After Kosovo's assembly election in November 2007, deputies of the assembly unanimously declared Kosovo to be an independent and sovereign state.

About seventy states recognized Kosovo's independence so far [3]. Serbia and Russia and other state's denounced it as illegal. On October 8, 2008 the UN General Assembly adopted a resolution (63/3) to request the court's advisory opinion on the issue [4].

Jurisdiction and discretion

The international court of justice (ICJ) advisory jurisdiction is due to the text of article 96 of the UN charter and article 65 (1) of the ICJ statute.

The debate about court's jurisdiction arose several arguments with some participant in Kosovo advisory opinion. The next problem was about the court discretion. These problems will be considered in the following sections.

Jurisdiction in charter and statute with respect Kosovo case

For each request of advisory opinion, the court must first consider whether it has jurisdiction, and whether there is any reasons to use its discretion and decline to exercise its jurisdiction.

The power of the court to give an advisory opinion is based upon article 65, paragraph 1, of its statue, which provides that "the court may give an advisory opinion on any "legal question" at the request of "whatever body" may be authorized by or in accordance with the charter of United Nations (UN) to make such a request [5].

Therefore the conditions for request of an advisory opinion are: "request by an organ duly authorized to seek this opinion under the charter, requested question must be a legal one, except General Assembly or the Security Council , that question should be one arising with the scope of the activities of the requested organ [6].

It is for the court to satisfy itself that request comes from an authorized organ. The GA is authorized by article 96 of the charter, which provides that:

"1-the General Assembly or the Security Council may request the international court of justice to give an advisory opinion on any legal question" [7].

The only condition mentioned in this article is that,

the question asked by GA must be a "legal" one. Although the court sometimes in the past mentioned in its jurisprudence that there must be a relationship between the question asked and activities of GA [8]. This assertion is far from being clear. Firstly, the court didn't expressly state that the existence of such "relationship" is a legal condition for the valid seizure of the court by the GA. Its ambiguous statement and reasoning however leave the door open to this interpretation [9].

Secondly and more fundamentally, the notion of "activities" undertaken by GA in relation to the question or, more generally, the "competences" of the GA, in the same sense as the "activities" referred to by article 96 Para 1 of the UN charter [10].

Interpretation the notion of "activities" in this latter sense is practically tenable not only, as explained below, in light of the final purpose of the advisory jurisdiction of the court, but also given more concretely, the considerations upheld by the court immediately following its ambiguous assertion. Those considerations indeed directly refer to article 10 and 11 of the UN charter [11], that is, articles concerned with the functions and powers of GA [12]. The court did not refer at that stage to the "concrete activities" conducted by GA in relation to Kosovo situation. It (sensibly) did so, as detailed below, only in the context of its discretionary power. As a result, the wording used by the court could give rise to interpretations where by the distinction between Para 1 and Para. 2 of article 96 fade away.

This may be supported by the jurisprudence to which the court referred when making its ambiguous assertion, and notably by its considerations upheld in the nuclear weapon case [13]. Indeed, how could such purpose be achieved if the question asked does not fall within the "competence" of the requesting organ? Having said that, one must acknowledge that the "competences" of the GA are so extended and extendable that it is unlikely that question could not be linked to any of such competences. In the Kosovo case, the court easily mentions the competences to which the question was related to, mainly maintenance of international peace and security [14]. Because article 11, Para 2 of the charter has provided the GA with competence to discuss "any question" relating to the maintenance of international peace and security brought before it by any member of UN, and subject again to limitation of article 12 [15].

Indeed, according to such provision, the GA cannot make any recommendation with regard to a dispute or situation concerning with the maintenance of international peace and security as long as the SC is effectively exercising its responsibility with respect to that dispute or situation, unless the SC so request. In wall case, the court mentioned that the requirement of UN GA resolution be in accordance with article 12 Para. 1 of the UN charter clearly appeared as a legal condition for a valid seizure of the court by the GA [16]. This requirement seems reasonable since the respect of the rule pertaining to the balance of powers between the GA and the SC is particularly important in order to give full legitimacy to the court's opinion. To solve this problem raised, the court by reference to its jurisprudent in wall case stated:" article 12 Para. 1 was not applicable here because the request for an advisory opinion could not be considered as a "recommendation"" [17].

In Kosovo case, the court (contrary to wall case) stopped its reasoning here, because it focused (only) at "concrete activities" conducted by GA in relation to the Kosovo situation and not the competence of this organ as may be interpreted as having done when analyzing its jurisdiction. Although such requirement seems to go beyond what is provided under article 96 of UN charter. It has been submitted that this requirement would actually be relevant in light of the final purpose of the advisory jurisdiction of the court and that such purpose would also justify that the link between the question and the activities (in the meaning of the "concrete activities") of the requesting organ be only discussed, as the court did in this case, in relation to the discretion (rather than the jurisdiction) of the court.

Discretion

The fact that the court has jurisdiction does not mean that it is obliged to exercise it. Article 65, Para 1, provides that "the court may give an advisory opinion" [18].The word may imply that court has a discretionary power to decline of giving its opinion even if the conditions of its jurisdiction are met [19].

The aim of discretion is protection of the integrity and the court's judicial function and its nature as the principal judicial organ of UN [20].

The court is always mindful of the fact that its answer to any request for an advisory opinion, "represent its participation in the activities of UN, which in principal should not be refused except the occasions where there is "compelling reasons" to do so [21]. Some arguments arose in Kosovo case in this respect which are as following:

"Consent of an interested state, motives behind the request, lake of useful purpose in GA resolution 63/3 and its legal effect, as Kosovo situation is very much linked to an actual dispute, the request must be asked by the sc ratter than GA, and political consequences of the court opinion".

Application of these arguments in Kosovo case

In course the Kosovo advisory proceeding, several states have called upon the court to exercise its discretionary in order to refuse to answer the GA

request in this case. In this section, I will consider their argument and the answers which in my opinion are justified.

First argument was about interested parties consent. Firstly, Serbia as a part of this opinion was itself main sponsor of the CA resolution. Secondly, since the statehood of Kosovo was the subject matter of the dispute, it would be difficult for the court, and states for that matter, to alledge that the consent of Kosovo would have been required to answer the request of advisory opinion.

Thirdly, although the request was also aimed at providing clarification as to whether the recognition of Kosovo as a independent state by others could have seen as a violation of international law, only very few states actually voted against this request [22].

Second argument concerns about the motives behind this request. This matter very much linked to the fact that the request related to an actual dispute. They believed that the request did not have as its principal objective to assist the UN GA in the exercise of its functions but in effect only served the interest of Serbia. Because Serbia in its statement at the time of adoption of resolution 63/3 by GA stated:

"It seems this opinion to be politically neutral, yet judicially authoritative, guidance to many countries still deliberating how to approach unilateral declaration of independence in line with international law" [23].

The court answer (as before) was that, "its opinion is given to the organ which has requested it. Therefore the motives of individual state which sponsor or vote in favor of GA resolution for requesting the court opinions are not relevant to the courts discretion [24].

The third argument concerns about GA resolution 63/3 and lack of the legal effects of this opinion for GA. The court answered this problem by reference to its jurisprudence and stated:

"It is not for the court itself to purport to decide whether or not an advisory opinion is needed by requesting organ of UN for the proper performance of its functions. That organ (in this case the GA) has the right to decide for it self on the usefulness of an opinion in the light of its own needs" [25].

The forth argument is about political consequences of the court opinion. The court stated in several time before that "there are no evident criteria by which it can prefer one assessment to another" [26].

The fifth and the most important argument were about respective roles of the SC and the GA in relation to the situation of Kosovo. As some participants and judge s like Tomka, Keith, Sepulveda- Amor and Bennouna believed that this request for advisory opinion must put to the court by the SC rather than GA [27]. Their reasons was that situation in Kosovo had been the subject action by the SC for a decade, in exercise of its responsibility for maintenance of international peace and security [28]. The GA also adopted 20 resolutions on the situation of Kosovo which five of them were about human rights [29] and 15 of them concerning the financing of UNMIK [30]. At the time of issuing the unilateral declaration of independence of Kosovo (2008), the GA feels it necessary to create a new agenda item for a request of an opinion form the ICJ. This decision of the GA in those circumstances seems justified, because of the fallowing reasons:

1. According to well- established jurisprudence of court, the only "compelling reasons" should lead the court to refuse its opinion is in response to a request falling within its jurisdiction [31].

2. Articles 10 and 11 of the charter confer upon the GA a very broad power to discuss matters within the scope of the activities of the UN, including questions relating to international peace and security [32].

3. As court made it clear in its jurisprudence, article 24 of charter [33] refers to a primary, but not necessarily exclusive competence [34].

4. The fact that the situation in Kosovo is before the SC and it has exercised its authority in chapter VII does not preclude the GA from discussing any aspect of Kosovo situation, including the unilateral declaration of independence. Because of the limits which the charter place upon the GA to protect the role of the SC are contained in article 12 of charter [35]. This article restricts the power of the GA to make recommendations following a discussion, not its power to engage in such a discussion.

More over this article does not bar all actions by the GA in respect of threats to international peace and security before the sc. The court considered this matter in detail in its jurisprudence and stated:

"there has been an increasing tendency over time for the GA and the SC to deal in parallel with the same matter concerning the maintenance of international peace and security and observed that in these occasions the SC has tended to focus on the political aspects of such matters and the GA has taken a broader view, considering also their humanitarian, legal, social and economical aspects of the same matter" [36].

5. As the court has mentioned before, GA resolution 337 A (V), ("uniting for peace") provides for the GA to make recommendations for collective measures to restore international peace and security in any case where there appears to be "a threat to the peace, breach of the peace or act of aggression and the sc is unable to act because of lark of unanimity due to veto right of the permanent members [37].

6. The purpose of the court's advisory jurisdiction is to enable organs of the UN and other authorized bodies to obtain opinions from the court which will assist them in their future exercise of their functions. The court cannot determine what steps the GA may

wish to take after receiving the advisory opinion or what effect this opinion may have in relation to those steps. So GA is entitled to discuss the unilateral declaration of independence of Kosovo without trespassing on the power of the SC.

8. The interpretation and application of a decision of one of political organs of the UN is, in the first place, the responsibility of the organ which took the decision. But the court as the principal judicial organ of the UN has also frequently been required to consider the interpretation and legal effects of such decisions.

It has done so both in the exercise of its advisory jurisdiction [38], and in the exercise of its contentious jurisdiction [39].

There is nothing incompatible with the integrity of the judicial function in the court under taking such a task. Because the court observes where, as here, the GA has a legitimate interest in the answer of the court to GA question, the fact that, this answer may turn, in part, on a decision of the SC is not sufficient to justify the court in declining to give its opinion.

The court concludes from the forgoing reasons that "there are" no compelling reasons" for it to decline to exercise its jurisdiction in respect of the GA request before it [40].

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27- See: www.ICJ.org.

28- The sc first action in relation to the situation in Kosovo was adoption of resolution 1160 (31 Mar 1990), which was followed by other resolutions namely: resolution 1199 (1998), 1203 and 1239 (1999). On 10 June 1999, the sc adopted resolution 1244, which authorized the creation of an international military presence and an international civil presence (the united nations interim administration mission in Kosovo, "UNMIK"), and laid down a framework for the administrative of Kosovo, By resolution 1367 (2001) the sc decided to terminate the prohibitions on the sale or supply of arms established by Para. 8 of resolution 1160 (1998). The sc has received periodic reports from the secretary- special envoy on the activities of UNMIK. Although the declaration of independence of the Kosovo was discussed by sc but it took no action in this respect so far.

29- Resolutions: 49/204, 50/190, 51/111, 59/130, and 53/164.

30- Resolutions: 53/241, 54/245A, 54/245B, 55/227A, 55/295, 57/326, 58/305, 59/286A, 59/286B, 60/275, 61/285, 62/262, 63/295, 64/279.

31- Case concerning Legal consequences of the construction of a wall in the occupied Palestinian territory ,Op. Cit, PP. 156- 160, Para's. 51- 54.

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Change in intramuscular and intermuscular neural adaptation after resistance training in trained college athletes

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Abstract: The purpose of this study was to investigate the intramuscular and intermuscular neural adaptation after resistance training in trained college athletes. The results indicated that, progressive resistance training significantly ($P<0.01$) increased MVC and integrated electromyography (IEMG) of MG muscle. There were, however, a significant decrease ($P<0.01$) in integrated electromyography (IEMG) of TA muscle. It was concluded that, with strength improvement in trained limb agonist muscle activation increased whereas antagonist muscle coactivation decreased. Consequently intermuscular neural adaptation (decrease of muscle coactivation) and intramuscular neural adaptation include increase of motor unit recruitment, firing rate and firing duration, does occur in trained athletes when a new training stimulus is implemented.
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Keywords: Strength training; electromyography; gastronomies

1. Introduction

It is well established that physical activity that incorporates high muscle tensions, i.e., heavy-resistance strength training, can lead to an increase in maximal contractile muscle force. However, the specific mechanisms responsible for this adaptation are not fully known [1]. The increase in maximal contraction force may not solely be explained by increases in muscle cross-sectional area or volume. Rather, an increased "neural drive" to the muscle fibers contributes to the training-induced increase in maximal contractile force, even in the absence of increases in muscle size. Thus not only muscle size and muscle phenotype but also neural innervations are important determinants of maximal contractile muscle strength in vivo [1-2]. Physical training can stimulate both neurological and muscular adaptation [3-4], which can result in an increase in muscular force. Of the two, neurological adaptations to training are less understood especially in trained athletes. Although the effect of resistance training on muscle morphology has received considerable examination, less is known about the specific neural mechanisms responsible for the training-induced increase in maximal muscle strength [1]. Nonetheless, support for such change was demonstrated [3-5]. The specific neural adaptation that occurs is thought to be the result of improvements in intramuscular and intermuscular coordination. The intramuscular adaptations include motor unit recruitment, firing rate, synchronization of firing, and stretch reflex input; and the intermuscular adaptations include activation of synergists and co-contraction of antagonists [3]. Numerous reports exist of the morphological changes in human skeletal muscle induced by resistance training. Such changes include increases in anatomical muscle cross-sectional area [6-7], steeper muscle fiber pen nation angles [6], physiological muscle fiber area and increased percentage 2A fibers with a corresponding decrease in 2X fibers [8-9]. Likewise, the neural adaptation induced by resistance training has been addressed with the use of integrated electromyography (EMG) as an indicator for a change in efferent neural drive. Several investigators have reported increases in integrated EMG after resistance training and reported more synchronous motor unit impulses on electromyography (EMG) after resistance training when compared with pre-training patterns [1-2,7], although not consistently demonstrated in all studies [10-11]. In addition, Rutherford and Jones [12] suggested that training establishes new neural pathways that increase the coordinated activation of the muscle groups involved in a particular muscle action. Furthermore, Carolan and Cafarelli [13] proposed that reduced antagonistic co-contraction after isometric training of the leg extensors may be responsible for the greater torque-producing capabilities of the agonist muscle. It has also been proposed that training elicits alterations in the excitatory and/or inhibitory input, so that a greater inflow of impulses reaches the motor neuron of the working muscle [2,14]. Recent investigations have attempted to determine whether training induces greater motor neuron activation by monitoring EMG activity over the course of a resistance training program. Some studies have indicated that EMG activity increases with training [2,7,14-16],

supporting the hypothesis of increased neural activation and Others, however, have reported no such change [10-11,17]. Aagaard et al [1] reported that some of this disparity may be explained by the inherent methodological constraints associated with the recording of surface muscle EMG during maximal voluntary contraction (MVC). It is commonly thought that these neurological improvements occur during the initial stages of training, after which a gradual crossover to muscular adaptation occurs [18-19]. This would lead to the assumption that neurological change does not occur in the trained athlete, at least not to any significant degree. However, it is also logical to assume that, when a different training stimulus is introduced, additional neurological and muscular adaptations may occur. Because of lake and discrepancy in the research literature to evaluating such a possibility therefore, the purpose of this study was to investigate the intramuscular and intermuscular neural adaptation after resistance training in trained college athletes.

2. Methods

In order to determine if a new training stimulus would stimulate neural adaptation in previously trained men college athletes, the subjects were divided into 2 groups, experimental (EXP) and control (CON). The only alteration between CON and EXP group was that the experimental group engaged in a form of resistance training on plantar flexor muscles, whereas the control group did not. Various techniques have been employed to identify whether neural mechanisms are involved during a training program; however, we chose surface EMG as a simple, noninvasive measure of neural activation. Data were collected prior to initiating the new training protocol and at the end of 8 weeks.

Thirty male college student athletes(body mass 74.03 ± 5.41 kg, height 175.80 ± 6.31cm, age 22.33 ± 1.26 yr and sport history =3.8 1.31years, means ± SD) with no neuromuscular dieses history served as subjects for this experiment. The subjects were randomly assigned to either CON (n=15) or EXP (n=15) in an even distribution. The subjects were carefully informed about the design of the study with special information on possible risks and discomfort that might result, and subsequently signed an informed consent document prior to the start of the study. Before the first test, limb dominance was noted by the subject's preference in kicking a ball and taking off in a single-legged hop [20].

The young men were football athletes who were in an off-season. Subjects in the training group trained for 8 weeks. During training, they trained the plantar flexor muscles. Two types of progressive strength training, standing one-leg calf raises and Donkey calf raises, were performed 3 times a week for 8 weeks. One-leg calf-raises were carried out with each subject standing with their knee and hip joints secured in a neutral position. Each subject raised the heel of the training side from the neutral position to 30° plantar flexion in the sagittal plane, while supporting a barbell on their shoulders. In order to performance of Donkey calf raises exercise the subject stood with toes on the edge of a calf board (approximately 3-5 inches in height). The subject bent forward at the hips until the torso was parallel to the floor, and he stabilized the body by holding onto a piece of equipment. In this position he allowed the heels to drop as far as comfortably possible below the level of the toes. Then he raised the torso as high as possible on the balls of the feet. Once the top of the movement is reached he slowly lowered the heels as far below the level of the toes as possible, returning to the starting position [21]. Weight bag according to each subject 1RM put onto their back. Strength-training exercise consisted of 3 sets of 10-12 repetitions at 70-75% of the one-repetition maximum (1-RM) with a rest period of 1-2 min between sets. The 1-RM was tested by the formula (1RM = Bar Wtkg ÷ 1-(0.02 × Reps) [22] on the 1st day of every week during training for 8 weeks, and the intensity was adjusted to maintain a progressive resistance training stimulus. Before testing, each subject warmed up for 5 min with aerobic, low-resistance ergometer cycling and static stretching of the leg muscles [20]. One specific warm-up set of 15 repetitions was performed for each exercise at an intensity of 45% of the 1 RM. For control of the repetition speed compensatory acceleration technique was used [22]. A single investigator supervised each training session. The total time for carrying out the training program was 30 to 45 min for each session.

Each subject completed three trials of MVC, with each trial separated by a minimum of 180 s. In the MVC test, the subject was asked to increase force gradually for 2 s, and thereafter to try to keep the maximal force for approximately 2 s [23]. Before the pre-training measurements, each subject was familiarized with the equipment used and the procedures involved in the experiment. The force of plantar flexion was measured by a load cell (Lafayette, USA) placed between the metal base plate and force lever plate. The force signal from the load cell was amplified through a DC amplifier (32528, Lafayette, USA). Each subject sat on a seat, and was positioned at 80° hip flexion and 10° plantar flexion, with the knee at the neutral position. A belt was used as a support to keep the hip joint, knee joint, and thigh unchanged during testing [23]. The foot was also tightly secured by two straps to keep the ankle joint unchanged. Arms were folded in front of the

chest [20]. Verbal encouragement was given during the exercises. Hip, knee and ankle postures were measured using a SG110 and SG150 twin axis goniometer, (DataLog, Model P3X8, Biometrics, UK). The electrogoniometer was attached with double-sided (medical) adhesive tape and secured with adhesive medical tape. Goniometry recordings were analyzed using the datalog ver.2.0a software analysis package.

The measurements of each parameter were performed at pre-training (PRE) and post-training (POST) in both the training and the control groups.

The surface EMG signals of muscle were recorded during MVC by way of surface bipolar electrodes. The skin surface was cleaned with alcohol and rubbed with sand paper. The index of good skin impedance condition was that the skin gets a light red color and for fixed skin impedance condition 5 minutes time was used [24]. The room temperature fixed at 25 ºC. Surface bipolar electrodes Ag-AgCl (Medicotest blue sensor, M-oo-s), with 6 mm contact diameter, and 1.5 cm inter-electrode space were placed at the tibialis anterior ~10 cm below the caput fibulae, and at the gastrocnemius medial heads ~7 cm below the caput fibulae (2). For avoid cable movement artifacts and minimized the risk of separating the electrodes from skin, the cable, pre-amplifier and electrodes fixed on the skin by regular tape and net bandages. In order to cooperate between MVC test and EMG measurement we used alarm of EMG device. A circle was drawn with a permanent marker around all the electrodes to ensure consistent relocation of the electrodes. The EMG signals were amplified differentially with an AC amplifier (gain 375), and band-pass filtering was set at both low pass (500 Hz) and high pass (10 Hz) cutoff filters (ME3000p8, Mega Electronic, Finland) and sent to a personal computer via an analog to digital (A/D) board with Sensivity 3 mV and Resolution: 2.95 mV (12-bit, 8 channels, Mega Electronic, Finland).

All signal processing was performed using markers of Megawin software (version 2.0; Mega Electronic, Finland). The signals were band-pass filtered from 10-500 HZ. The EMG data of the MVC trial was used to calculate the IEMG for 1- second time between 800 ms before and 200 ms after the peak.

Data are presented as mean (SD), and these were computed by standard methods. The changes to each variable during the experimental periods (PRE and POST) were analyzed using paired students t-test. The independent t-test was used to determine a significant differences between trained and control groups. A Statistical significance was set at the $P<0.05$ level. These statistical analyses were performed with SPSS software (SPSS 11.5, SPSS).

3. Results

The changes in MVCs, IEMG values obtained from the medial gastrocnemius (MG) (Agonist) and tibialis anterior (TA) muscle (Antagonist) at PRE and POST in the EXP and CON group are shown in Tables 1 and 2.

Table 1- Changes in variables at pre-training (*PRE*) and post-training (*POST*) in the EXP group, Values are means (SD). (*MVC:* Maximum isometric voluntary contraction, I*EMG:* integrated electromyography).

Variable	EXP group	
	PRE	POST
MVC (Kg)	2.61±22.31	2.7*±24.84
IEMG of (MG) muscle (μV·s)	66.89±1321.13	68.36*±1406.26
IEMG of (TA) muscle (μV·s)	52.23±917.51	49.04*±823.59

*Significantly different from PRE at $P<0.01$

Table 2- Changes in variables at pre-training (*PRE*) and post-training (*POST*) in the CON group, Values are means (SD). (*MVC:* Maximum isometric voluntary contraction, I*EMG:* integrated electromyography)

Variable	CON group	
	PRE	POST
MVC (Kg)	2.63±22.34	2.59±22.29
IEMG of (MG) muscle (μV·s)	69.1±1319.53	67.98±1317.26
IEMG of (TA) muscle (μV·s)	51.12±916.38	50.89±917.11

*Significantly different from PRE at $P<0.01$

Figures 1-3 show the percentage changes in plantar flexor MVC, IEMG of the MG muscle and TA muscle values at pre-training (PRE) and post-training (POST), in the EXP and CON group.

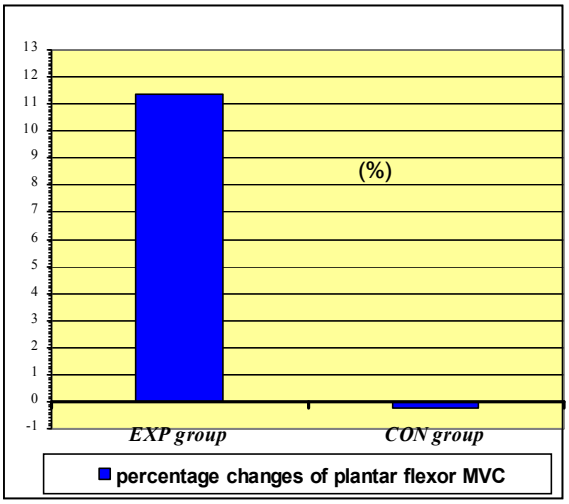

Fig 1- percentage changes in plantar flexor MVC at pre-training (*PRE*) and post-training (*POST*) in the EXP and CON group

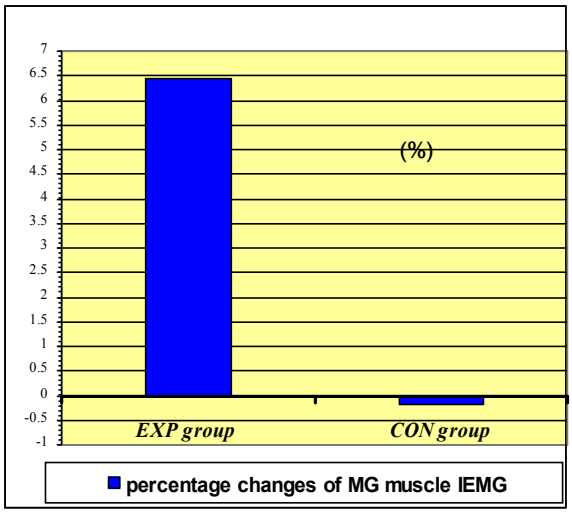

Fig 2- Percentage changes in MG muscle IEMG at pre-training (PRE) and post-training (POST) in the EXP and CON group

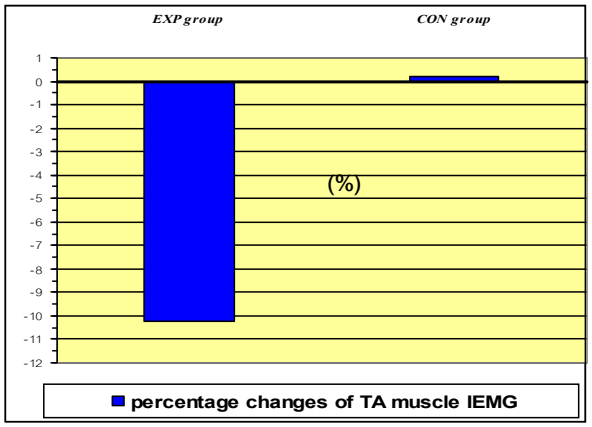

Fig 3- Percentage changes in TA muscle IEMG at pre-training (*PRE*) and post-training (*POST*) in the EXP and CON group

After training, MVC was significantly increased (11.34%, P<0.000) in EXP group but no significant change in the CON group. Percentage changes in plantar flexor MVC at pre-training (PRE) and post-training (POST) in the EXP and CON group was shown in figure 1.

The IEMG of the MG muscle significantly increased (6.44%, P<0.000) in EXP group after training whereas there was no significant change in the CON group. Percentage changes in MG muscle IEMG at pre-training (PRE) and post-training (POST) in the EXP and CON group was shown in figure 2.

The IEMG of the TA muscle significantly decreased (10.23%, P<0.000) in EXP group after training. For the CON group there was no significant difference in this variable. Percentage changes in TA muscle IEMG at pre-training (PRE) and post-training (POST) in the EXP and CON group was shown in figure 3.

The control subjects showed no significant changes for all variables throughout the experimental period, indicating that the changes observed in the training group were due to the new progressive resistance training programmer.

4. Discussions

The results of this study indicated a significant increase in plantar flexor MVC, MG muscle IEMG and a significant decrease in TA muscle IEMG after a new progressive resistance training in trained athletes. Some studies reported that resistance training increased agonist muscle EMG [1-2,7] although the others showed no significant change in this factor [10-11]. The reason for the discrepancies between the results of the present investigation and those of others examining EMG responses to training may be a function of differences in the mode, intensity, period of training and procedures used to analyze and quantify the EMG signal [11]. The present study used different contraction modes for isometric testing and for isotonic strength training. Isometric strength was assessed in order to avoid any positive learning effect, as subjects did not train with isometric contractions. Thus, differences in the muscle action used for testing purposes and the methods used to quantify EMG amplitudes could partly explain the conflicting results between the present study and other studies. Evetovich et al [11] reported that after training as individual muscle fibers enlarge; their positions under surface electrodes are altered. Therefore, it is possible that hypertrophy alone could have influenced the EMG signal. Garfinkel and Cafarelli [25], however, hypothesized that if electrode placement is constant, then the electrodes are detecting EMG over the same area of muscle membrane and, therefore, hypertrophy would not alter the EMG. Maximal iEMG changes after training may reflect the degree of electrical excitation of the underlying muscles and is affected by the number and size of motor units recruited, frequency of stimulation, and the synchrony of firing [15]. The interpretation of the increases in IEMG during maximal muscle actions after training is uncertain. Increases in IEMG can reflect increases in motor unit recruitment and/or motor unit firing rates [15]. Some studies that used the twitch interpolation technique with isometric muscle actions [26] have suggested that motor unit activation during maximal voluntary contractions before training is maximal. If this were the case, the increase in IEMG after training should reflect increased motor unit firing frequency, which may or may not cause greater force [25]. It is also

possible that increased surface area of hypertrophied muscle fibers could contribute to increased IEMG after training, but the relatively small muscle hypertrophy that occurred and the fact that muscle hypertrophy is not always accompanied by increased maximal IEMG [25] suggest that this is unlikely. Higbie et al [15] reported that the significant changes in strength after resistance training resulted from a combination of muscle hypertrophy and increased neural activation. However, it was not possible to precisely determine the relative importance of the two adaptations. They noted that based on the magnitude of the mean changes, and the correlations between changes in torque and changes in muscle hypertrophy and maximal IEMG, muscle hypertrophy and neural adaptations appeared to contribute approximately equally to the changes in strength after training. However, a substantial part of the strength change could not be accounted for by these two factors. Other studies have found that changes in muscle size or maximal IEMG after heavy resistance training are only moderately or poorly correlated with strength changes [27-29]. In the present study there was a significant decrease in TA muscle IEMG (antagonist) after training in trained athletes. Some studies found that after 8 weeks of training there was an increase isometric torque during resistance training that was not associated with a change in maximal activation of the agonist muscle but a decrease in EMG activity in the antagonist [10,12-13]. It is possible that the increase in maximal agonist muscle strength was due, in part, to a training-related decrease in co-activation of the antagonist muscles. It has been suggested, however, that this should result in greater agonist activation, and thereby cause increased agonistic EMG activity [10]. Rutherford and Jones [12] have suggested that changes in antagonist co-contraction are learned adaptations, whereas Carolan and Cafarelli [13] stated that the level of co-activation changes with training. The magnitude and the time course of the changes in the antagonist co-activation may be related to the types of action used, to the exercises utilized in the training and to the initial physical status of the subjects in terms of experience and skill in strength training. Nevertheless, the present result support the concept that strength training can lead not only to the increased activation of the agonist muscle in trained athletes but training-induced learning effects in terms of reduced co-activation of the antagonist muscle also plays a role enhancing the net force production of the agonists. The extent to which reduced co-activation of the antagonist is mediated by mechanisms in the central nervous system or associated also with peripheral neural control, especially during various dynamic actions, is difficult to establish [30]. Changes in co-activation could be a learned adaptation manifested as an improvement in coordination or skill. Reducing antagonist co-activation requires no conscious effort and therefore is likely mediated by mechanisms in the central nervous system. It has been suggested that co-activation is facilitated by Renshaw cell firing, which inhibits the Ia inhibitory inter-neurons by excitation of the Ib inter-neurons from the Golgi tendon organs or by direct descending motor pathways. Attenuation of any or all of these pathways would reduce co-activation. Because adaptations in antagonist co-activation do not account for all the nonhypertrophic increases in quadriceps MVC, the possibility of additional changes occurring elsewhere in the neuromuscular system must be entertained [13].

Pucci et al [31] noted that an increase in motor unit recruitment may have contributed to the increase in surface EMG activity and may have occurred independently of increases in mean motor unit firing rates. Reductions in co-activation have been proposed to be a contributing factor to the increase in agonist MVC with training. They reported that the small changes in activation and co-activation observed in their studies are not likely to be sufficient to solely account for the large increases in MVC force during training. It is possible that other neural adaptations may also have occurred. Such adaptations include changes in the control of the synergistic muscles [12,32] and motor unit firing rate synchronization [33]. The argument for neural factors being involved in strength increases hinged on increases in muscle activation observed in the surface electromyogram. At maximal efforts this adaptation was interpreted to mean a more complete recruitment of the entire motor unit pool or increased motor unit firing rates [31]. Also it has been suggested that training establishes new neural pathways that increase the coordinated activation of the muscle groups involved in a particular muscle action.

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The aerial parts of *Stachys schtschegleevii* Sosn. as hydroalcoholic extract has antibacterial Activity on Multi-Drug Resistant Bacterial Isolates in Comparison to Ciprofloxacin

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Abstract: Several *Stachys* species have been used traditionally for their health benefits in various countries. About 34 species of *Stachys* are found in Iran. This plant possesses various medicinal properties, but there have been performed a few studies on the antibacterial effects of *Stachys schtschegleevii*. The aim of the present study was evaluation of the antibacterial activity of *Stachys schtschegleevii* on 100 Multi Drug Resistant isolates of *Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella spp.* and *Escherichia coli*. *Stachys schtschegleevii* samples were collected from the fields of northwestern Iran. The flowering aerial parts of the plant were cut, and powdered, and then extracted by maceration at room temperature for 72 hours. Bacterial isolates were collected from clinical specimens from several wards of educational hospitals in Urmia, Iran during a 12 months period. The susceptibility of isolates to *Stachys schtschegleevii* extracts was determined using a broth microdilution method. Considering the wide application of ciprofloxacin in treatment of bacterial nosocomial infections, the antibacterial effect of ciprofloxacin on isolates was also determined. All the multi-drug resistant bacterial isolates were sensitive to different concentrations of *Stachys schtschegleevii* hydro-alcoholic extract. The most sensitive bacterial isolates to *Stachys schtschegleevii* extracts were *P.aeruginosa*, however, 69% of isolates were resistant to ciprofloxacin. The results demonstrated that this herbal drug could represent a new source of antimicrobial agents, for the control of hospital acquired infections. However, more adequate studies must be carried out to verify the possibility of using it for fighting these bacteria in human body infections. Additionally, antifungal activity of this plant must be studied along with its antibacterial effect.
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Keywords: herbal medicine, ciprofloxacin, resistant bacteria, antimicrobials, hospital acquired infections

1. Introduction

Resistant bacteria to the antimicrobials turned to be one of the most challenging problems of the recent century. The slogan of WHO in this year, is an evident support for this allegation. In this case, herbal medicines seem to be a new step toward fighting them. Iran is a country rich of medicinal plants and most of these plants are currently used by people as home remedies. Studying on antimicrobial activity of these herbs considered as noteworthy.

Stachys is a genus of shrubs and annual or perennial herbs. It belongs to family *Labiatae* that is considered as medicinal plant as an antipyretic, antiseptic, antispasmodic, astringent, carminative, diuretic, febrifuge, hypotensive, stomachic, styptic, tonic, vermifuge and antibacterial. It is taken orally as a medicinal tea in the treatment of fevers, diarrhea, sore mouth and throat, internal bleeding, and a tonic of liver and heart (Raymond M. Harley, 2004).

Stachys was named by Linnaeus in *Species Plantarum* in 1753 (Carolos, 1753).

The name is derived from the Greek word σταχυς (*stachys*), meaning "an ear of grain" (Umberto Quattrocchi, 2000) and refers to the fact that the inflorescence is often as a spike.

The plant's most useful constituents are betulinic acid, D-camphor, delphinidin, hyperoside, manganese, oleanolic acid, rosmarinic acid, rutin, ursolic acid, as well as various saponins and tannins. Also Preliminary phytochemical studies showed that the aerial parts of the genus *Stachys* contain flavonoids, which may be responsible for their antibacterial activity (M Saeedi, 2008).

Stachys are widely distributed in tropical and subtropical countries. Estimates of the number of

species in the genus vary from about 300 (Raymond M, 2004), to about 450 (David.J, 2008). 34 of them are found in Iran, of which 13 are endemic (Mozaffarian VA., 1996 and Rechinger KH. 1982). *Stachys schtschegleevii* is a native plants in Iran (Rechinger KH. 1982).

In the Iranian traditional medicine the extracts of the aerial parts of *Stachys schtschegleevii* (traditionally named *Poulk*) have been used in infectious, rheumatic and inflamamatory respiratory diseases (Shamsali Rezazadeh, 2005).

Since there are limited studies on the antibacterial effects of *Stachys schtschegleevii* extract on multi-drug resistant (MDR) bacteria, we evaluated its hydro-alcoholic extract on clinically resistant bacteria.

Resistant Gram-positive pathogens, such as *Staphylococcus aureus* have become a serious problem in clinical medicine. *S. aureus* is an organism with several virulent factors and resistance mechanisms at its disposal. It is also a significant cause of a wide range of infectious diseases in humans. *S. aureus* often causes life-threatening and long-lasting infections like bacteremia, endocarditis and pneumonia (Kanafani and Fowler 2006)**.**

Acinetobacter baumannii is a gram-negative opportunistic bacillus. It is found in many hospital environments and can colonize in human body in the hospital environments. The combination of its environmental colonization and its very high resistance to antimicrobials renders it as a successful nosocomial pathogen. The MDR strains of A. *baumannii* are often spread and cause outbreaks throughout hospital wards. *A. baumannii* cause a wide range of clinical complications, such as pneumonia, septicemia, urinary tract infection, wound infection, and meningitis, especially in immunocompromised patients (Nordmann, 2004).

Pseudomonas aeruginosa is an opportunistic pathogen found as a part of the normal flora of the human skin (Larson and Ramphal, 2002). In immunocompromised host, *P. aeruginosa* can colonize and infect the burn and wound sites. It can rapidly disseminate from the wounds into other organs via the bloodstream and can produce severe infections such as endotoxic shock (Dale et al., 2004). Antibiotics are generally ineffective against most serious infections especially burn wounds infections caused by *P. aeruginosa*. The problems of treating these infections is frequently complicated by antibiotic resistance, a problem that is increasing in the recent years.

Klebsiella spp. are a group of Gram negative rods and they can cause different kinds of infections especially in a hospital setting. They are resistant to numerous antibiotics. Their resistance to antibiotics restricts the choice of antibiotics for therapy (Keynan and Rubinstein, 2007).

Hospital acquired urinary tract infections account for 35-45% of the nosocomial infections (Kamat et al, 2009). *E. coli* is the main agent of this disease. Antibiotic therapy is the gold standard of treating such infections; however, long-term therapy may result in many side-effects and cause selection of resistant bacteria. Thus, we need new treatments that could replace antibiotic therapy (Jazani et al 2007).

In respect of high resistance of nosocomial isolates of mentioned bacteria to antimicrobials, introducing of the new antimicrobial agents against these kind of microorganisms is one of the most important goal in treatment of such infections (Perez et al., 2007).

In this study we evaluated the antibacterial activity of hydro-alcoholic extract of aerial parts of *Stachys schtschegleevii* on 100 Multidrug resistant isolates of *Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella* and *E. coli*.

2. Material and Methods

For data collection, the junior medical students were grouped into several groups. Then, the information gathered from ethno-pharmacologists, herbal-drug sellers and rural native-healers, from different regions of Iran, especially Northwest, Southwest, Central and Northern provinces. All data collected, were summarized for every species. For each repeated report of a certain indication we added “a point” to the specification of that plant. If the number of every reported indication was more than 7-15 times we reported that indication or pharmacological effect in our final report in this article. We thought that, the higher frequency of the reports of an activity or indication, may mean the most reliable applications of that plant in Iranian traditional medicine. We report here, the plants used in Iranian traditional herbal medicine with cold nature.

3. Results

Extract preparation: *Stachys* samples were collected from the fields of West Azerbayjan province, the northwestern Iran, and identities were confirmed by the Botanist. The flowering aerial parts of the plant were cut, chopped, dried and powdered. The powders were extracted by maceration at room temperature for 72 hours. The hydro-alcoholic extracts were combined and concentrated to yield a dried powder, and it was kept in refrigerator for all experiments (Garjani et al, 2009).

Bacterial strains and culture media: A total of 100 isolates *of Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella* and *E. coli* (20 isolates from each kind) were collected from clinical specimens of different wards of educational hospitals in Urmia, Iran during a 12 months period between April 2006-2007. The isolates were further processed by the standard methods to identify as the *Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella* and *E. coli* isolates (Baron and Finegold, 1990). The susceptibilities of isolates to different antibiotics were tested using agar disk diffusion method and Multidrug resistant isolates was selected for further experiments. Isolated bacteria were maintained for long storage on skimmed milk medium (BBL) by adding 10% glycerol in -60°C, cultures were maintained for daily use on Nutrient agar (BBL) slants on 4°C. The Muller Hinton Agar (MHA) and Muller Hinton Broth (MHB) medium (Pronadisa) were used for detection of antibiotic resistance of isolates. *Acinetobacter calcuaceticus* PTCC 1318, *Enterococcus faecalis* ATCC29212, *Pseudomonas aeruginosa* ATCC27853, *Pseudomonas aeruginosa* PAO1, *E.coli* ATCC25922, *Klebsiella pneumoniae* ATCC10031, *Staphylococcus aureus* PTCC1112 and *Staphylococcus aureus* ATCC25923 have been used as reference strains.

Determination of antimicrobial activity of *Stachys* extracts: The susceptibility of isolates to *Stachys* extracts was determined using a broth microdilution method based on CLSI guidelines. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Stachys* extracts for isolates were determined in Muller-Hinton Broth (MHB; Oxoid) medium (Jazani et al, 2009) (Papadopoulos et al., 2006). 10 mg of *Stachys* powder was dissolved in 1000 µL of Dimetylsufoxide (DMSO, Sigma). The initial concentration of *Stachys* powder in the first tube contains MHB was 500 µg/mL. This was used to prepare serial doubling dilutions over the range 500-3.9 µg/mL. 1.5×10^6 inoculums of the isolates were added to each concentration in MHB. A tube containing growth medium without *Stachys* extracts and an un-inoculated tube were used as a positive and negative growth control respectively. Antibacterial activity was measured by determining MICs and MBCs. The MIC was the lowest concentration of essential oil that resulted in a clear tube. Ten microlitres from each tube was spot-inoculated onto Nutrient Agar (NA) and incubated overnight at 37 °C to determine the MBC. The highest dilution that inhibits bacterial growth on nutrient agar after overnight incubation was taken as MBC (Baron and Finegold, 1990; Papadopoulos et al., 2006). Experiments were performed at least three times and the modal value selected.

Determination of antimicrobial activity of ciprofloxacin: Considering to the wide application of ciprofloxacin in treatment of bacterial nosocomial infections, the antibacterial effects of ciprofloxacin on isolates also determined and the effectiveness was compared with *Stachys* extracts. Ciprofloxacin powder was kindly provided by Exir pharmaceutical company, Tehran, Iran. The pure content of active ciprofloxacin was 96% in the provided powder. For determining of the bacterial isolates sensitivity to ciprofloxacin, classic broth dilution susceptibility test were used (Sahm and Weissfeld, 2002). MIC and MBC of isolates to ciprofloxacin were determined. The initial concentration of antibiotic in the first tube was 500µg mL^{-1}, this solution was diluted serially in 8 steps. 1.5×10^6 inoculums of the isolates were added to each concentration of ciprofloxacin in MHB. A tube containing growth medium without ciprofloxacin and an un-inoculated tube were used as a positive and negative growth control respectively. *In vitro* resistance was defined as MBC of 4 or more µg mL^{-1} for bacterial isolates (Chaudhry et al., 1999).

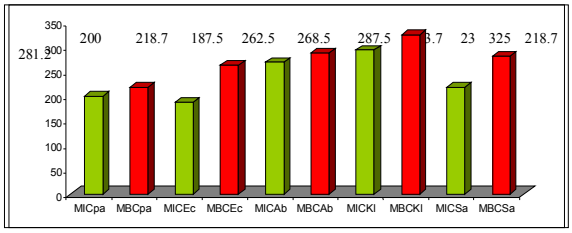

Fig 1: Antibacterial activity of *Stachys* hydroalcoholic extract against 100 nosocomial isolates of multi drug resistant gram negative and gram positive bacteria.

Pa: *Pseudomonas aeruginosa*, Ec: *E. coli*, Ab: *Acinetobacter baumannii*, Kl: *Klebsiella Sp*, Sa: *Staphylococcus aureus*. MIC: Minimum Inhibitory Concentration, MBC: Minimum Bactericidal Concentration.

3. Results:

A total of 100 multi-drug resistant isolates with nosocomial origin of gram negative and gram positive bacteria were collected from clinical specimens submitted to the educational hospital clinical microbiology laboratories of selected hospitals in Urmia, Iran. The Sensitivity of bacterial isolates to *Stachys* hydroalcoholic extract has been shown in Figure 1**.**

Also the MIC and MBC of *Stachys* hydroalcoholic extract against standard bacterial strains has been shown in Table 1.

Table 1: The MIC and MBC of *Stachys* hydroalcoholic extract against standard bacterial strains.

Standard Bacterial isolates	*Stachys* hydroalcoholic extract(µg/mL)
Acinetobacter calcuaceticus PTCC 1318	MIC= MBC=125
Enterococcus faecalis ATCC29212	MIC= MBC=250
Pseudomonas aeruginosa ATCC27853	MIC=125, MBC=250
E.coli ATCC25922	MIC= MBC=125
Klebsiella pneumoniae ATCC10031	MIC= MBC=125
Staphylococcus aureus PTCC1112	MIC= MBC=250
Staphylococcus aureus ATCC25923	MIC= MBC=125
Pseudomonas aeruginosa PAO1	MIC=125, MBC=250

The Sensitivity of bacterial isolates to ciprofloxacin has been shown in Fig 2. 69 isolates (69% of all isolates) were resistant (MBC $\geq$ 4 or µg mL^{-1}) and the other isolates were sensitive to ciprofloxacin (MBC $\leq$ 4 µg mL^{-1}) (Fig 2).

4. Discussions and Conclusion

The antimicrobials have increasingly been ineffective against most serious infections by multi drug resistant bacteria, treatment of these infections is frequently complicated by antibiotic resistance, a problem that is increasing in recent years, so introducing of the new antimicrobial agents against these kinds of bacteria is one of the most important goals in treatment of such infections. However there are limited studies on investigation of the antibacterial effects of *Stachys schtschegleevii* extract on multi drug resistant bacteria.

Saaedi et al. performed a study to evaluate the antimicrobial activity of the methanol extracts of dried flowering aerial parts of *Stachys byzantina* , *S. inflata* , *S. lavandulifolia* and *S. laxa* (Labiatae) using the disc diffusion method and determination of (MIC) values against *Staphylococcus aureus*, *Streptococcus sanguis*, *Escherichia coli*, *Pseudomonas aeroginosa*, *Klebsiella pneumoniae*, *Aspergilus niger* and *Candida albicans*. The antimicrobial activities and MICs of *methanol extract of S. byzantine* (14.1%), *S. inflate* (14.3%), *S. lavandulifolia* (10.1%) and *S. laxa* (10.6%) exhibited concentration-dependent antibacterial activity against bacteria tested. The methanol extracts were more active against *Streptococcus sanguis* and *Staphylococcus aureus*. Their preliminary phytochemical studies also showed that the aerial parts of the genus *Stachys* contain flavonoids, which may be responsible for their antibacterial activity. (M Saeedi, 2008)

Chitsaz et al. studied aqueous and methanolic extracts of *St. schtschegleevi* on *Staphylococcus aureus*, *Streptococcus pyogen*, *E coli* and *Pseudomonas aeroginosa*, using well diffusion method and determining MIC and MBC by standard macro dilution NCCLS method.

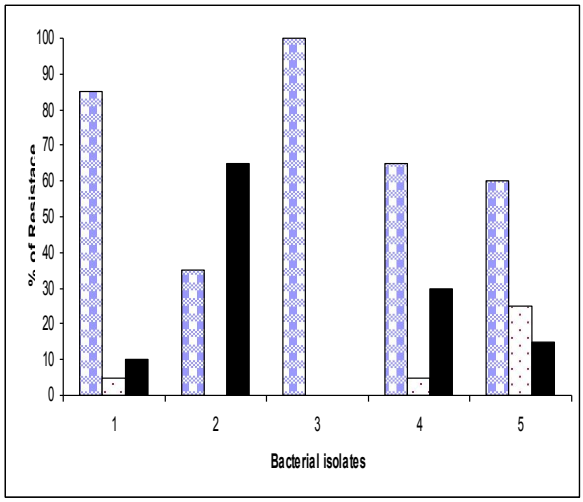

Figure 2: The rates of resistance to Ciprofloxacin for 100 clinical isolates of multi-drug resistant bacteria. Resistant (checked), Intermediate (spotted), Sensitive (black). 1: *Staphylococcus aureus,* 2: *E. coli,* 3: *Acinetobacter baumannii,* 4: *Klebsiella Sp.* And 5: *Pseudomonas aeruginosa.*

By well diffusion method, inhibition zone for *Staphylococcus aureus* and *Streptococcus pyogen* was 23.67 mm and 17.30mm, respectively. The corresponding values of MIC and MBC were 6.25, 12.5 for *Staphylococcus aureus* and 12.5, 12.5 for *Streptococcus pyogen*, respectively.

According to the results of their study, the aqueous extract had no anti-bacterial activity against tested bacteria and methanolic extract was not active against tested gram-negative bacteria (Chitsaz, 2006). However, in our study the most sensitive bacterium to *St. schtschegleevii* was *Pseudomonas aeroginosa*.

In another study, Helen D. Skaltsa *et al.* studied the antimicrobial activity of essential oil of eight *Stachys* species, including *St. alopecuros* (L.) Bentham., *St. scardica* (Griseb.) Hayek, *St. cretica* L. subsp. *cretica*, *St. germanica* L. subsp. *heldreichii*

(Boiss.) Hayek, *St. recta* L., *St. spinulosa* L., *St. euboica* Rech. and *St. menthifolia* Vis. The studied species were of bacteria including: *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 35210), *Bacillus subtilis* (ATCC 10907), *Bacillus cereus* (clinical isolates), *Micrococcus flavus* (ATCC 10240), *Staphylococcus epidermidis* (ATCC 2228), as well as five fungi, including: *Aspergillus niger* (ATCC 6275), *Penicillium ochrochloron* (ATCC 9112), *Epidermophyton floccosum* (clinical isolates), *Candida albicans* (clinical isolates) and *Trichophyton mentagrophytes* (clinical isolates). The results of the study showed the tested essential oils had better activity against bacterial species than fungi, but *St. scardica* was the most active on both bacteria and fungi. *Pseudomonas aeruginosa* (Pa) was the most resistant strain. In addition, *Pseudomonas aeruginosa* (Pa) was the most resistant strain to all essential oils (Helen D. Skaltsa**,** 2003).

This is in contrast with our study's result, in which the most sensitive bacteria to *St. schtschegleevi* Sosn. was *Pseudomonas aeruginosa* (Pa).

In another similar study in Turkey, G Dugler and C Aki investigated antimicrobial activities of the ethanol extract of the leaves of *Stachys pseudopinardii*, *R. Bhattacharjee* (Lamiaceae), against *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 7064, *Staphylococcus aureus* ATCC 6538P, *Escherichia coli* ATCC 10538, *Proteus vulgaris* ATCC 6899, *Salmonella typhimurium* CCM 5445 and *Pseudomonas aeruginosa* ATCC 27853, as well as *Candida albicans ATCC 10239*, *Debaryomyces hansenii* DSM 70238, *Kluyveromyces fragilis* ATCC 8608 and *Rhodotorula rubra* DSM 70403, by disc diffusion and microdilution methods. In their study, all extracts showed strong antibacterial activity against *Bacillus cereus* ATCC 7064, with an inhibition zone of 25.0 mm (MIC=16 µg/mL) and (MBC=32 µg/mL). *Debaryomyces hansenii* DSM 70238 was among the most susceptible of the yeast cultures, with an inhibition zone of 17.0 mm and MIC=32 µg/mL and minimum fungicidal concentration (MFC) of 32 µg/mL. The extract exhibited moderate activity against the other test microorganisms (G Dugler and C Aki,2009).

As the study showed, the ethanol extract of the leaves of *Stachys pseudopinardii*, had significant antimicrobial activity and it may be useful in the treatment of mentioned infections. Therefore, we can test our extracts against fungi, too.

In the present study all the multi-drug bacterial isolates were sensitive to different concentrations of *Stachys* hydroalcoholic extract, the most sensitive bacterial isolates to *Stachys* extracts were *P. aeruginosa* isolates (Fig 1). Also *A.calcuaceticus* PTCC 1318, *E.coli* ATCC25922 and *S.aureus* ATCC 25923 and *Klebsiella pneumoniae* ATCC10031 were the most sensitive strains among the standard isolates (MIC= MBC=125) (Table 1), however clinical isolates showed high resistance to ciprofloxacin (Figure 3).

In the present study results showed that the *Stachys* hydroalcoholic extract possessed antibacterial effect against all multi-drug resistant bacteria isolates, furthermore, beside the confirmation of the popular use, the obtained results demonstrate that this herbal drug could represent a new source of antimicrobial agents, for the control of hospital-acquired infections. However, studies that are more adequate must be carried out to verify the possibility of using it for fighting these bacteria in human body infections.

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Weather types, their frequency and relation with rainfall in west of Iran

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Abstract: Over time, various places experience different weather; there is even possible and quite tangible some different kinds of weather in a season. In this study, an automated synoptic classification (T S I) with meteorological data of six synoptic stations in the west of Iran has been used, which are exposed to Mediterranean and Sudan systems, in different seasons of the year to categorize each day by its particular category. The used variables include cloudiness (12GMT), maximum and minimum daily temperature, Dew point (12GMT), maximum and minimum daily humidity, dry temperature degree (12GMT), daily rainfall, and u and v components of wind in 1961-2004 periods. After elimination of days lacking data, by creating a P-type matrix, the correlated variables were identified and selected in form of independent components as an objective technique by analyzing principal components, and then a homogeneous collection was extracted from days of record periods in each station under name of weather types by hierarchical cluster analysis (AHC). Afterwards, through evaluating rainfall amount in every weather type and its related days using cluster analysis (CA), isohyets types were identified in west of Iran. Accordingly, 4 isohyets groups were identified in 26 weather types, including high rainfall, very high rainfall, medium rainfall, and very low rainfall. Occurrence time frequency of weather types in stations of the region was studied in terms of presence number of each weather type throughout the each year and as repeated occurrence in each individual day of statistical year (number of presence of each weather type in 1 Jan of all years then 2 Jan and so to 29 Dec) and subsequently their seasonal activity was determined.
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Keywords: Weather type; rainfall; Iran; climate; meteorological

1. Introduction

In fact, Air masses refers primarly to source region but Weather types refers to time formation of those variables or components (days clustering of each station) which play an important role in forming a certain type of its climate. In other words, there is a systematic collection of main elements of weather at different times, and they reflect similar specifications. Repeating these species of weather expresses climate type of a location, and this plays an important role in managerial decisions in long-term behaviorology. By considering the thermal and humidity homogeneity of air masses during their presence in a region in horizontal, it can provide conditions such that meteorological variables reflect special amounts based on their specifications of bottom surface, from geographical latitude and longitude, topography, height from sea level, and distance from humidity sources, at which if different classes of these elements are included in manufacturer climate during time, they can identify weather types. Identification of weather types and studying their frequency of occurrence for identification of climate location is necessary in order to explain reaction of geographical location to air mass duration and there is an appropriate assessment from behavior of surface level. Therefore, any actions consistent with showing different weather types are very important in order to better understand the climate-regulating managerial decisions. The purpose of this study is automated weather type classification in stations in west of Iran, during those days of year when they are exposed to westerly winds and Mediterranean and Sudan systems. Several researches have been carried out in this field, and the related ones will be considered.

Barrera (2002) classified weather types about the flood-generating rainfalls during small frost (1840-1870) in Catalonia. He collected useful information from 10 old cities in Europe and used them in order to study the meteorological floods relation, and then reconstructed synoptic daily surface data with pressure. He used data series of daily meteorological information in Barcelona during 1870-2002 in order to study changes and time of flood events. In total, 62 meteorological events were identified.

The researchers have determined 6 weather types, including southern air flow, northern air flow, western-oriented, cyclonic, anti-cyclonic, and eastern air flow. They found that in the four seasons, southern air flow had assigned the highest abundance with 31 flood events, so it can provide floods in every season and field.

Cheng et al. (2004) executed an automatic synoptic classification method in order to predict snow in Ottawa, Ontario. This study was based on data including temperature, dew point, surface pressure, total overcastting, u and v components of vector wind directions, and snowfall occurrence, as well as 6 hours of upper-atmosphere data in 6 atmospheric levels with network density 2.5˚×2.5˚, including temperature, relative humidity, geopotential height, vertical wind speed, and speed of west-east and south-north winds in all days of winter from 1958 to 1990. Using principal component analysis and cluster analysis based on hierarchical agglomerative style, the input matrix data consisting of 240 variables resulted in synoptic temporal index (TSI), and as a result, 18 principal components were achieved which explained 92% of variance.

In this analysis, thermodynamic variables set aside the most contribution, including temperature and dew point with variance 36%. By clustering factor scores, 13 synoptic groups were obtained with synoptic weather type, which allocated 85% of total days. Finally, these researchers matched these weather types with snowfall days, and then they compared frequency of days with snowfall; meantime they fitted 4 weather types with these conditions by regression model. Afterwards, they used them for predicting conditios in 1991-2001 decade, which showed an appropriate compliance with the initial series of data.

Christensen and Brayson (1966) tested the ability of principal components analysis in order to classify weather types. In their study, they used 15 meteorological parameters with two times of daily observations including dry temperature, wet temperature, more overcasting, pressure, u and v components, etc. They entered the obtained results into multiple regression analysis by analyzing principal components with Varimax rotation, and determined weather types by selecting correlation threshold as 0.7.

The studied data had a four-year duration (1955 -1958) for January, and five year duration (1954 -1958) for July. Each type of features was studied according to mean and quarter of each variable, and finally the synoptic situation of the classified days for each season was compared. Then, 107 out of 124 evaluated days for January were grouped in 25 types or classes, and 17 days were also identified as days of transition. Subsequently, they carried out this process for July.

Davis and Kalkstein (1990) divided days of 1984 into same groups based on six meteorological elements. They studied synoptic index and called the obtained calendar and time classification as climate periods, and then they expanded the above-mentioned index to place of synoptic index. The selected ingressive of 90 types was classified, and after that they reduced them to 10 principal weather types as the results of their research.

Kerschner (2005) studied weather type's rainfall climatology in eastern Alps Mountain, in order to test the feasibility of weather type classification and regional analysis of rainfall synoptic climatology.

He used surface air flow 500 hPa and anomaly quarters of daily mean on a network with 25km cell distance as principal data and for classification. The classiufication includes 24 directional classes and 5 classes for weak or mixed airflow patterns. Then, he extracted daily rainfall data of the network from internet and analyzed the possibilities greater than 1mm daily rainfall in weather types. He found that anti-cyclonic weather types usually have too many negative anomalies in all seasons, and cyclonic types with too many positive anomalies are specified in core of certain regions such as northern Alps margin.

Littmann (2000) presented an experimental classification of weather types in Mediterranean basin, and subsequently studied internal relationship of the types with rainfall. He identified major and essential elements of synoptic large-scale patterns on synoptic maps during 1992-1996 for 1338 days as pressure cells.

He organized pressure centers data according to their presence as binary, 1 or 0. Then, he carried out cluster hybridization by analyzing hierarchical cluster and Euclidean distance square technique and Ward algorithm, and finally separated 20 clusters with ability to explain 69% of variance. He identified rainfall area in a 1º×1º network consisting of monthly total rainfall, by using PCA analysis, in order to study weather type's relationship with Mediterranean rainfall, which resulted to detection of 10 components with explanation of variance 92%.

He proofed a significant internal communication by using Chi-square test ($\chi 2$), and noted that rainfall patterns in Mediterranean regions core (components 1 and 2) have been well explained by synoptic types.

Mc Gregor and Bamzelis (1995) studied synoptic typing and its application to air pollution in Birmingham, England. They used 11 variables in their research, including overcasting, wet and dry temperature, dew point, vapor pressure, atmospheric pressure, relative humidity, visibility, total radiation, and u and v components, as well as related data to pollution consisting of sulfur dioxide, nitrogen dioxide, ozone, nitric oxide, carbon monoxide, and particles smaller than 10 microns (PM10).

Afterwards, they achieved four components with variance 81.2% by analyzing principal components with P matrix configuration and Varimax rotation, which were determined to include hygrothermal, fog, westerly, and cloud. The researchers determined 6 weather types by hierarchical cluster analysis, and then interpreted synoptic charts with center of each type. They also studied contamination by using representative days of each weather type.

From mean concentration of O3, NO2 and PM10, they found that weather mass 5 was important. They also found that fourth weather mass (number 4) was important from PM10, NO2 and NO. Furthermore, the most frequent of events with severe pollution has typically anti-cyclone nature, which has a poor ventilation system. Also, western cyclone streams have a better air quality, altuough there has been increased O3 levels due to vertical transmission from upper troposphere.

Muller (1977) achieved 8 types for New Orleans, by assessing daily surface weather map sets in the United States. Next, weather types were changed to 3 environmental indexes in order to analyze the relationship between climate and environmental reactions:

1) Shower index with wet, cloudy, and windy air;
2) Continental polar index with cooler air and lower dew point; and
3) Tropical marine index with warmer and very desirable weather.

This technique was used to periodically study local-effect Gold quest region.

Sfetsos et al. (2004) attempted to identify representative days in synoptic climatology studies. They carried out actions in order to identify groups of days with common characteristics using data of two complete years, applied prediction model in Meteorological Institute of Norway (DNMI), analyzed the components based on findings, carried out differential cluster analysis, and observed information plus obtained data from the model. This study was performed in a region with 280,000 square kilometers area between southwest coast of Norway and Shetland Islands.

Stone (1989) developed a similar method to TSI. He used principal components analysis (PCA) for meteorological surface data in Brisbane, Queensland, as well as Varimax rotation and correlation matrix, in order to obtain orthogonal components. Then, he used Ward cluster technique in order to identify large weather types. Finally, he identified 25 hybrid types and 63 sub-types for a 15-year period from 1967 to 1981.

In this article, we have used Temporal Synoptic Index to group the days of six stations in the west part of Iran (Kalkstein and Corrigan 1986). We have applied some weather type to each group of these days, and conducted an experiment regarding the raining amount on these types' grouping. We used multivariate statistical methods to better perceive the weather types temporal conformity and behavior on each other. This process helps us enhance our awareness toward the raining behavior of each weather type and the effect of atmospheric systems in the west part of Iran. Besides, considering the aerology characteristics, the diverse presence of weather types, and their comparisons in our case study make the present ecological conditions on the satiations and areas more manifest.

2. Material and Methods

For this research, 10 meteorological variables were used which describe the heat and moisture properties of the atmosphere including cloudiness (12GMT), maximum and minimum daily temperature, Dew point (12GMT), maximum and minimum daily humidity, dry temperature (12GMT), daily rainfall, and u and v components (12GMT) of wind in six synoptic stations located in west of Iran, including Kermanshah, Sarpolzahab, Ilam, Khorramabad, Sanandaj, and Nozheh in 1961-2004 period. map distribution of the stations is presented in Fig. 1 and their general specifications are presented in Table 1.

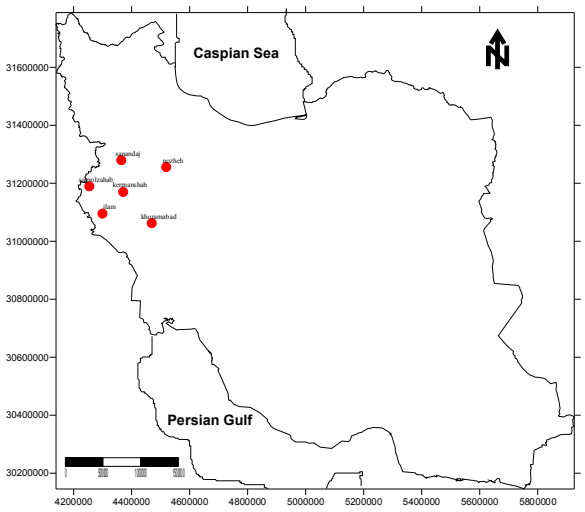

Fig 1- Location of selected stations in Iran

A matrix was formed from the mentioned variables with P mode, after initial preparation of data series and elimination of days without data. Standardization of data was subsequently performed in order to scale data; and then for removing repeated effects of correlated variables, initial variables were

reduced to fewer principal components with explanation of most variance percent, by using principal components analysis (PCA) and an eigenvector technique, based on Cattell test.

Table 1- General specifications of the stations

Station	longitude	latitude	Altitude(m)
Kermanshah	47. 15	34. 35	1318. 6
Sarpolzahab	45. 87	34. 45	545
Ilam	46. 43	33. 63	1337
Khoramabad	48. 27	33. 43	1147. 8
Sanandaj	47. 00	35. 33	1373. 4
Nozheh	48. 72	35. 20	1679. 7

In the next stage, we used analysis hierarchical clustering (AHC) with Ward technique on factor scores, in order to separate days with homogeneous meteorological conditions (Green et al 1999). As a result, the station weather types and/or Temporal Synoptic Index (TSI) were identified. Meteorological characteristics of each weather type were studied via calculating mean important variables; then similar weather types of the stations were identified based on mean rainfall by using cluster analysis, and the isohyet groups were determined. For more information about the presence of weather types during year and studying their occurrence in any given day of year in statistical period, the frequency of occurrence of each type was calculated by using a script in Matlab software.

3. Results

After constructing the databases and their standardization, based on Eigen vectors, variables of each station were reduced to variables with the highest explanatory variance, by principal components analysis (PCA). Results of Eigen vectors are presented in Tables 2-7, and decision was made about number of retained components. Figs. 2-7 show the importance and power of principal variables based on their rate variability.

Table 2- Eigenvalues of Kermanshah station

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Eigenvalue	4.781	1.650	1.115	0.851	0.697	0.492	0.223	0.129	0.058	0.005
Variability %)	47.810	16.501	11.147	8.511	6.966	4.922	2.227	1.288	0.582	0.047
Cumulative %	47.810	64.310	75.457	83.968	90.934	95.856	98.083	99.371	99.953	100.000

Table 3- Eigenvalues of Sarpolzahab station

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Eigenvalue	4.931	1.619	1.081	0.839	0.730	0.496	0.170	0.084	0.045	0.005
Variability (%)	49.307	16.193	10.810	8.394	7.297	4.963	1.702	0.842	0.445	0.046
Cumulative %	49.307	65.500	76.311	84.705	92.002	96.965	98.667	99.509	99.954	100.000

Table 4- Eigenvalues of Ilam station

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Eigenvalue	5.23	1.58	0.99	0.78	0.71	0.47	0.12	0.09	0.03	0.00
Variability (%)	52.25	15.78	9.88	7.85	7.08	4.69	1.22	0.90	0.32	0.04
Cumulative %	52.25	68.03	77.91	85.76	92.84	97.53	98.75	99.65	99.96	100.00

5- Eigenvalues of Khoramabad station

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Eigenvalue	4.82	1.69	1.01	0.94	0.66	0.51	0.21	0.11	0.05	0.00
Variability %)	48.19	16.86	10.13	9.44	6.59	5.09	2.09	1.05	0.51	0.05
Cumulative %	48.19	65.05	75.19	84.63	91.22	96.30	98.39	99.44	99.95	100.00

6- Eigenvalues of Sanadaj station

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Eigenvalue	4.68	1.65	1.13	0.89	0.71	0.48	0.26	0.12	0.07	0.00
Variability (%)	46.83	16.54	11.31	8.87	7.06	4.85	2.64	1.18	0.68	0.04
Cumulative %	46.83	63.37	74.68	83.55	90.61	95.46	98.10	99.28	99.96	100.00

7- Eigenvalues of Nozheh station

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Eigenvalue	4.59	1.69	1.10	0.91	0.71	0.53	0.29	0.12	0.06	0.00
Variability (%)	45.92	16.88	11.04	9.06	7.13	5.27	2.85	1.23	0.59	0.04
Cumulative %	45.92	62.79	73.83	82.89	90.02	95.29	98.14	99.37	99.96	100.00

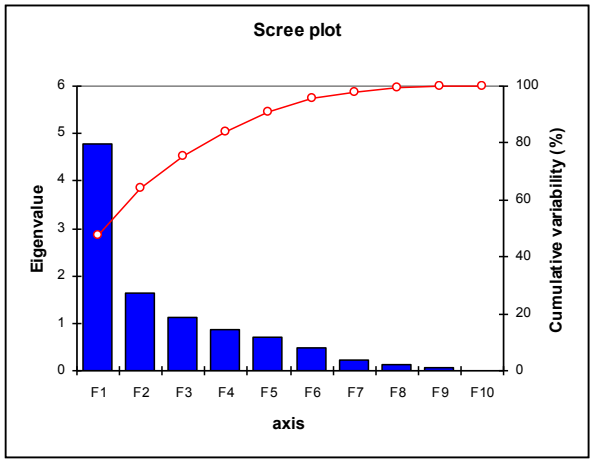

Fig 2- Screeplot of Kermanshah station

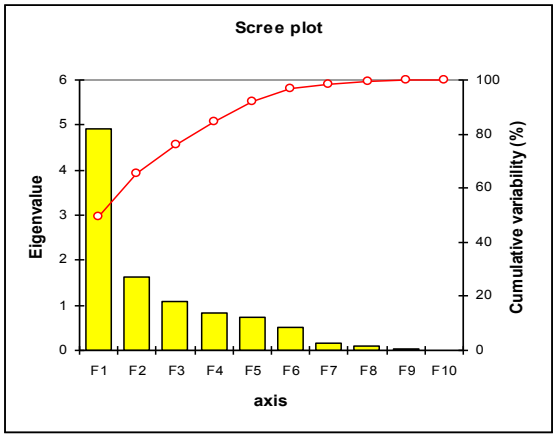

Fig 3- Screeplot of Sarpolzahab station

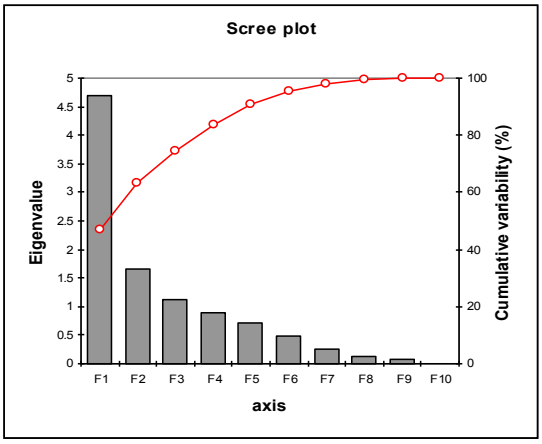

Fig 6- Screeplot of Sanandaj station

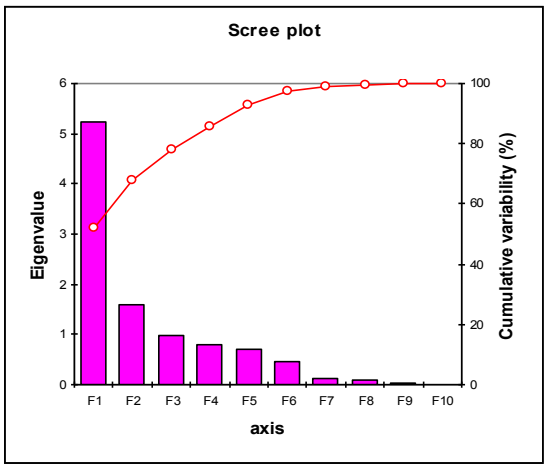

Fig 4- Screeplot of Ilam station

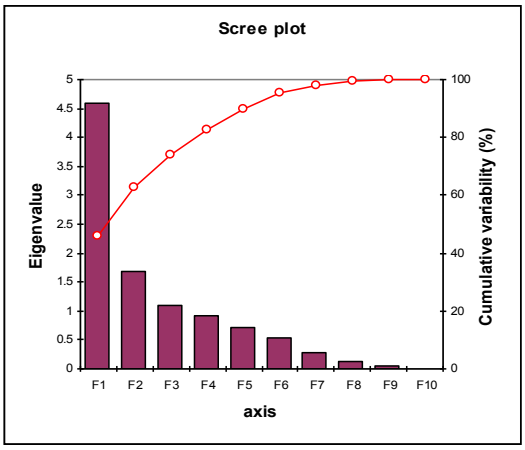

Fig 7- Screeplot of Nozheh station

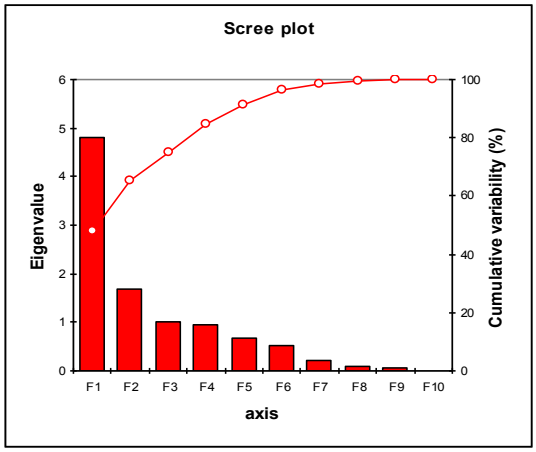

Fig 5- Screeplot of Khoramabad station

In all stations, there is homogeneity in occurance of the components characteristics, through selecting the first three principal components, which explain approximately 80% of the variance. In this case, the first, second, and third components reflect thermal, humidity, and windy characteristics, respectively.

Weather typing was performed in each station by objective method and factor scores of components, hierarchical cluster analysis, as well as using Akaic information criteria (AIC) for cutting dendrograms. Except Sarpolzahab and Khoramabad stations with 5 weather types, other stations had 4 weather types. The means of some types of meteorological characteristics are presented in Table 8.

Table 8- Meteorological characteristics of weather types in the stations

variables / Weather types	Mean daily cloudiness	Mean daily temp (C°)	Mean daily dew (C°)	Mean daily precipitation (mm)	Max daily precipitation (mm)	Min daily Precipitation (mm)	Total precipitation (mm)	Number of rainy days	Mean daily humidity (%)
Type1 kermanshah	0.8	24.2	0.8	0	7	0	80.7	977	24.8
Type2 kermanshah	3.6	3.3	-2.5	1.2	24	0	4749.3	1190	69.2
Type3 kermanshah	3.7	11.7	1.1	0.8	25	0	3846	1256	53.4
Type4 kermanshah	6.9	8	4.9	16.9	108	0	11323	669	81.5
Type 1 sarpolzahab	2.3	19.5	6.8	0.1	7	0	119.6	99	48.6
Type 2 sarpolzahab	0.5	30.2	6.7	0	4	0	19.4	16	26.5
Type3 sarpolzahab	2.6	9.6	2.5	3.3	9	0	5975	1371	65.4
Type 4 sarpolzahab	4.9	19.3	6.5	2.4	33	0	1289.8	249	50.4
Type 5 sarpolzahab	6	13	8.7	9.8	102	0	6536	554	77.2
Type1 ilam	3.5	9.2	0.1	1.3	31	0	3038.3	713	56.4
Type2 ilam	1	26.5	0.5	0	8.1	0	111.1	91	20.5
Type3 ilam	6.6	5.6	2.4	15.5	103	0	8263	505	81.4
Type4 ilam	1.1	12.7	-5.6	0	6	0	10.8	9	31.4
Type1 khoramabad	0.6	27.4	4.2	0	26	0	88	59	25.3
Type2 khoramabad	2.7	5.6	-1.7	3.4	8	0	9154	2043	62.9
Type3 khoramabad	1.8	16.5	2.6	0.1	13	0	348	209	43.8
Type4 khoramabad	4	13	3.7	1.4	28	0	4053	1087	57
Type5 khoramabad	6.7	8.1	5.1	12.2	71	0	16463.8	1295	82.4
Type1 Sanandaj	0.9	24.4	1.3	0	7	0	144.2	701	26.7
Type2 sanandaj	3.3	3.9	-4.3	0.4	19	0	1908.8	863	60.4
Type3 sanandaj	3.3	15.9	2.7	0.4	18	0	940.6	432	46.7
Type4 sanandaj	6.4	7.5	2.9	8.4	73	0	15548.4	1632	74.3
Type1 nozheh	5.9	2.8	-0.9	6.6	9	0	19516	2959	78.6
Type2 nozheh	1.1	22.4	1.8	1.8	9	0	9029	3093	30.2
Type3 nozheh	2.7	-2.5	-8.4	3.1	9	0	9046	2282	68.1
Type4 nozheh	3.3	12.2	-0.2	4.4	9	0	20552	4275	48.1

Considering the information in Table 8 obtained based upon calculations on data series of any type of weather, isohyet groups were separated by performing analysis hierarchical cluster and by aplying Ward technique to all weather types in the stations. Results of this classification are presented in Fig. 8 as dendrogram, and types of classes of isohyet weather types are shown in Table 9.

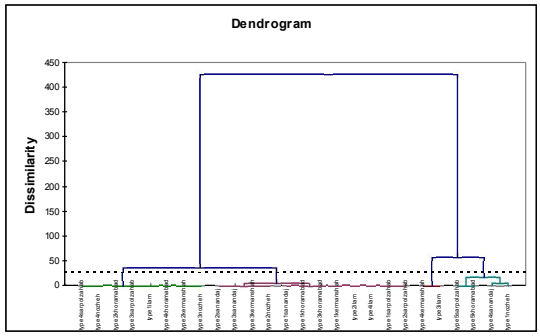

Fig 8- Clustering dendrogram of weather types

Table 9- Isohyet groups of weather types

	Rainy Groups (Classes)			
	Low	Medium	High	Very high
Weather types	Type (1) kermanshah			
	Type (1) sarpolzahab			
	Type (2) sarpolzahab	ype(2)kermanshah		
	Type (4) sarpolzahab	ype(3)kermanshah	Type(4)kermanshah	Type (5)khoramabad
	Type (2) ilam	ype(3)sarpolzahab	Type(3) ilam	Type (4) sanandaj
	Type (4) ilam	ype(5)sarpolzahab	Type (2)khoramabad	Type (1) nozheh
	Type (1) khoramabad	Type(1) ilam	Type (2) nozheh	Type (4) nozheh
	Type (3) khoramabad	/pe (4)khoramabad	Type (3) nozheh	
	Type (1) sanandaj			
	Type (2) sanandaj			
	Type (3) sanandaj			

After regulating relevant matrix, the occurrence of the repeatition of weather types was studied in every day of year and in the record period using a script (m-file) in Matlab software, in order to determine activity period of each weather type during year, or in other words, to identify their seasonal belonging, as well as their rise and disappearance. Expertized charts of each station are presented in Figs. 9-14.

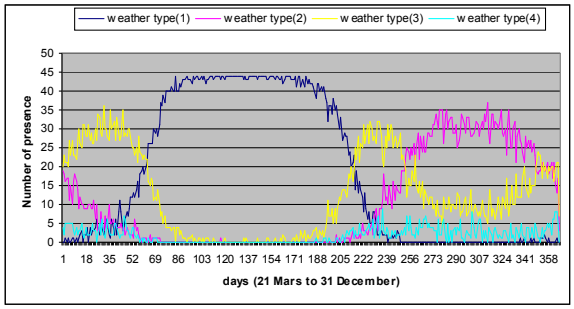

Fig 9- Presence frequency of weather types in Kermanshah station

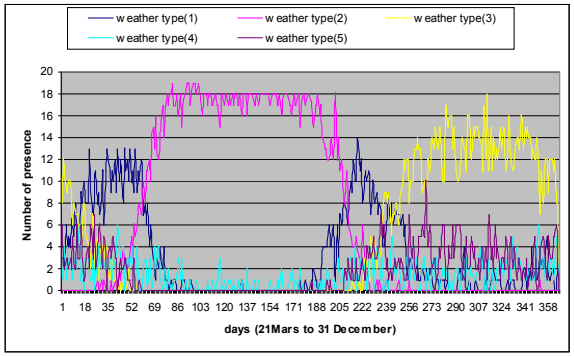

Fig 10- Presence frequency of weather types in Sarpolzahab station

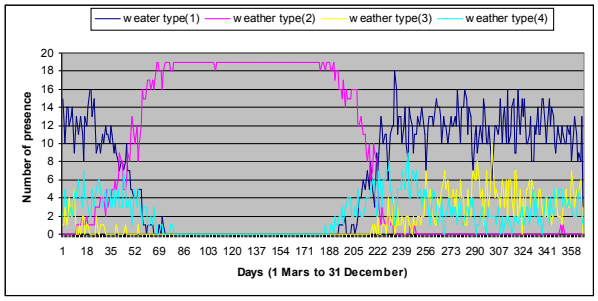

Fig 11- Presence frequency of weather types in Ilam station

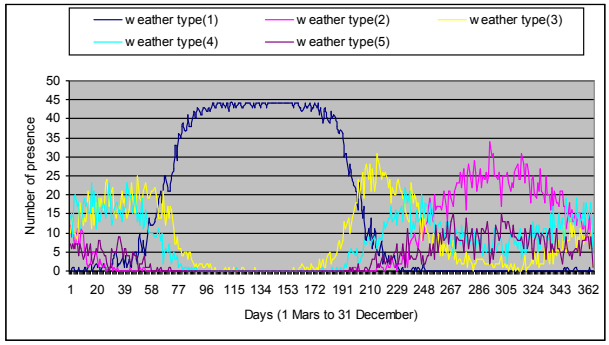

Fig 12- Presence frequency of weather types in Khoramabad station

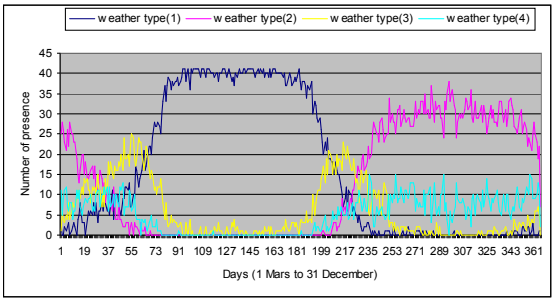

Fig 13- Frequency presence of weather types in Sanandaj station

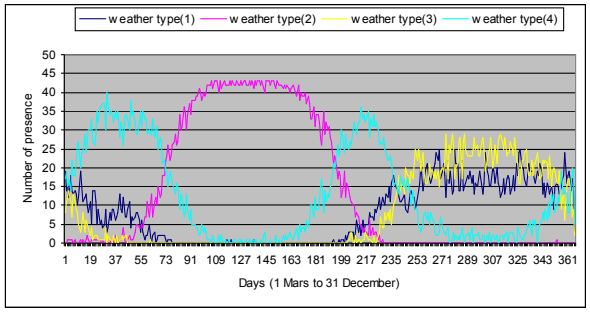

Fig 14- Presence frequency of weather types in Nozheh station

Activity periods of weather types include special seasons of a year.

Regardless of belonging each weather type of the stations to isohyet groups, the presence pattern of weather types can be divided into three categories using related frequency charts (Figs. 9-14):

1) Summer presence pattern: in this pattern, weather types have significant presence frequency in summer, and their presence is very small.

2) Bimodal pattern (spring and autumn): included types in this pattern are appeared in spring and autumn seasons, and their presence in summer and winter is very small.

3) Pattern without summer (three seasons): in this pattern, all stations have two defined weather types, and summer absence is one of charactristics of this pattern which differentiated it from the other patterns.

Results of classifying the patterns of presence frequency are presented in Table 10.

Table 10- Classification of presence pattern of weather types in west of Iran

Summer Pattern	Bimodal Pattern (spring & autumn)	Three seasonal Pattern (without summer)
		Weather type(2) kermanshah
	Weather type(3) kermanshah	Weather type(4) kermanshah
	Weather type(1) sarpolzahab	Weather type(4) sarpolzahab
	Weather type(4) ilam	Weather type(5) sarpolzahab
Weather type(1) kermanshah	Weather type(3)	Weather type(1) ilam
Weather type(2) sarpolzahab	khoramabad	Weather type(3) ilam
Weather type(2) ilam	Weather type(4)	Weather type(2)
Weather type(1)	khoramabad	khoramabad
khoramabad	Weather type(3)sanandaj	Weather type(5)
Weather type(1)sanandaj	Weather type(4) nozheh	khoramabad
Weather type(2) nozheh		Weather type(2)sanandaj
		Weather type(4)sanandaj
		Weather type(1) nozheh
		Weather type(3) nozheh

Weather typing in west of Iran showed that despite installment of stations in a geographical area (middle Zagros), there are other conditions, too, which cause specific differences in weather types of a station and between stations. These conditions can be studied from several points:

First, studying weather type inside stations and between them showed that they have a specified seasonal behavior; in other words, time factor influences the rise of special weather types because of forming regional synoptic systems including Siberian high-pressure, polar low-pressure, Azores high-pressure, Sudan, Ganges, and Mediterranean low-pressures, and western winds.

Second, by studying weather type in a station with similar absence of the rise characteristics but different meteorological data, the difference can be pointed between synoptic systems, different air masses, and different circulation flow patterns in sea level and 500 hPa levels.

Third, the weather type groups between stations which rise or are absent in similar seasons, in addition to previous reasons, can be added to specific conditions of each geographic location (topography, altitude).

Classification of weather types from amount of rainfall viewpoint revealed four stations with very high rainfall and high rainfall, which have presented specified seasonal behavior, and with high approximation, it means that they are absent in summer and they are present in other seasons.

Low rainfall group study showed that, the related weather types mainly are not activated in winter. Of course there are expectations in this field; meantime, Nozheh station has no low and medium rainfall types. Behavior of medium rainfall type confirms its absence in summer.

Research in recognition of weather types, especially in west of Iran, as one prone area with agriculture and horticulture and annual rainfall, is considered suitable for a more accurate understanding of the regional climate and it can help to manage climate change, especially in drought periods. Studying simultaneous occurrence of weather types and their relationship with circulation patterns with 500 hPa can elucidate the role of these patterns in shaping weather types.

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Considerations over the Biostratigraphy of Jeirud Formation

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Abstract: Biostratigraphy is the branch of stratigraphy which focuses on correlating and assigning relative ages of rock strata by using the fossil assemblages contained within them. Usually the aim is correlation, demonstrating that a particular horizon in one geological section represents the same period of time as another horizon at some other section. In location of Type section (Jeirud village – north east of Tehran), There are four members (A, B, C and D) and they are with a thickness of 760 meters. Among four members in Jeirud Formation, only member A is pertaining to late Devonian which has been separated, with a clastic unit, with a thickness of 20 meters from other member (B, C and D) in early Carboniferous. Lower boundary of member A is type of parallel unconformity with lilthostraigraphic units (Mila Formation) and is very index but there is no equal opinion about upper boundary. Also, based on advice from national stratigraphy committee in Iran, Jeirud Formation word is only equivalent to member of type section at the present which is representative of upper Devonian rocks in Central and western Alborz B, C, D members in Carboniferous can be compared with Mobarak Formation hence they do not have utility. This article aims to investigate the Considerations over the Biostratigraphy of Jeirud Formation, in addition to the use of basic biostratigraphy in different sediment types.
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Keywords: Biostratigraphy, Jeirud Formation, Sediment Types, Index Fossils.

1. Introduction

Biostratigraphy is a sub-discipline of sedimentary geology that relies on the physical zonation of biota, both in time and space, in order to establish the relative stratigraphic position (i.e. older, younger, same age) of sedimentary rocks between different geographic localities. Although the basic rules of biostratigraphic zonation were established in the late 18th to early 19th centuries in Europe (ultimately resulting in the development of the Relative Geologic Time Scale), the implementation of biostratigraphic techniques was in use in the United States during the early to mid-1800's. Some of the first geological surveys to be completed in the United States included those of the New York State Geological Survey. These surveys focused not only on New York's geological resources, but also emphasized the establishment of spatial and temporal relationships of stratigraphic units based on both lithologic and paleontologic composition. By the mid-1800's the New York Surveys had resulted in the development of a relative stratigraphic zonation based primarily on fossil distribution. New York localities are world famous for Cambrian through Devonian strata and fossils, but of particular importance to this website discussion is the contribution of the Ordovician rocks of central New York State to the establishment of a North American focused biochronology. The rocks found in the central New York Mohawk River Region, by definition of their fossil content, are now established as belonging to the Mohawkian Series of the Upper Ordovician Period. The following material focuses on key fossil taxa present in the Trenton Limestone, their distribution or occurrence within the overall succession of Upper Ordovician strata, and their role in the establishment of the Upper Ordovician time scale.

2. Biostratigraphy:

A Few Considerations

The goal of biostratigraphy is to use fossil occurrences within the rock record to establish correlations between time-equivalent rock strata as determined by the presence of a particular fossil species. Although the concept is generally straightforward, i.e. the presence of a specific fossil species in two geographic localities indicates the rocks containing the fossil specimens were deposited at about the same time, in practice biostratigraphic studies tend to be complex. The complexities of biostratigraphy result from aspects of the biology of the organisms including their environmental range, their evolutionary rates, as well as their tendency for

preservation and probability of observation by the biostratigrapher.

Ultimately, the most rapidly evolving or short-lived, yet wide-ranging fossil taxa make the best biostratigraphic markers for correlation. If a given taxa is both wide-ranging and evolutionarily short-lived, and if it is robust enough to be preserved in the fossil record, then the taxa is often referred to as an index fossil. An index fossil identified in the rock record would constrain the age of the rock within which it is contained to a very specific interval of time when the organism lived. Fossil taxa used in biochronologic investigations rarely satisfy all aspects of the ideal index fossil. That is, they often violate one or more of the following rules:

1) Must have a widespread distribution (fossils tend to be limited to a small region or are found only in a particular depositional environment as opposed to globally)

2) Must show rapid evolution (fossils change rapidly in preservable morphology so that distinctive identifiable species are easily recognized)

3) Must be present in substantial numbers (so that fossils can be observed by the biostratigrapher)

4), fossils should be robust mineralogically (so that depositional and diagenetic processes do not remove the fossils from the rock record).

Most often the best biostratigraphic markers or index fossils are taxa that live in the open water column either as free-floating plankton or as actively swimming nekton. Such organisms tend to be rapidly evolving, widely distributed and widely deposited. In contrast, benthic organisms which live on or very close to the seabottom, tend to be less widespread, fewer in numbers, and are typically found only in particular environments. Nonetheless, nektonic, planktonic, and benthic forms can be used to establish relative biostratigraphic age zonations. (http://www.mcz.harvard.edu, 2011)

3. Biostratigraphy

Biostratigraphy is the branch of stratigraphy which focuses on correlating and assigning relative ages of rock strata by using the fossil assemblages contained within them. Usually the aim is correlation, demonstrating that a particular horizon in one geological section represents the same period of time as another horizon at some other section. The fossils are useful because sediments of the same age can look completely different because of local variations in the sedimentary environment. For example, one section might have been made up of clays and marls while another has more chalky lime stones, but if the fossil species recorded are similar, the two sediments are likely to have been laid down at the same time.

Ammonites, graptolites, archeocyathids, and trilobites are index fossils that are widely used in biostratigraphy. Microfossils such as acritarchs, chitinozoans, conodonts, dinoflagellate cysts, pollen, spores and foraminiferans are also frequently used. Different fossils work well for sediments of different ages; trilobites, for example, are particularly useful for sediments of Cambrian age. To work well, the fossils used must be widespread geographically, so that they can occur in many different places. They must also be short lived as a species, so that the period of time during which they could be incorporated in the sediment is relatively narrow. The longer lived the species, the poorer the stratigraphic precision, so fossils that evolve rapidly, such as ammonites, are favored over forms that evolve much more slowly, like nautiloids. Often biostratigraphic correlations are based on a fauna, not an individual species, as this allows greater precision. Further, if only one species is present in a sample, it can mean that (1) the strata were formed in the known fossil range of that organism; (2) that the fossil range of the organism was incompletely known, and the strata extend the known fossil range. For instance, the presence of the fossil Treptichnus pedum was used to define the base of the Cambrian period, but it has since been found in older strata. (Gehling, 2001) Fossil assemblages were traditionally used to designate the duration of periods. Since a large change in fauna was required to make early stragtigraphers create a new period, most of the periods we recognize today are terminated by a major extinction event or faunal turnover.

The first reef builder is a worldwide index fossil for the Lower Cambrian
(Sengor, 1990; Beydoun, 1991)

Concept of stage

A stage is a major subdivision of strata, each systematically following the other each bearing a unique assemblage of fossils. Therefore, stages can be defined as a group of strata containing the same major fossil assemblages. French palaeontologist Alcide d'Orbigny is credited for the invention of this

concept. He named stages after geographic localities with particularly good sections of rock strata that bear the characteristic fossils on which the stages are based.

Concept of zone

In 1856 German palaeontologist Albert Oppel introduced the concept of zone (also known as biozones or Oppel zone). A zone includes strata characterised by the overlapping range of fossils. They represent the time between the appearance of species chosen at the base of the zone and the appearance of other species chosen at the base of the next succeeding zone. Oppel's zones are named after a particular distinctive fossil species, called an index fossil. Index fossils are one of the species from the assemblage of species that characterise the zone. The zone is the fundamental biostratigraphic unit. Its thickness range from a few to hundreds of metres, and its extant range from local to worldwide. Biostratigraphic units are divided into six principal kinds of biozones:

- Taxon range biozone represent the known stratigraphic and geographic range of occurrence of a single taxon.
- Concurrent range biozone include the concurrent, coincident, or overlapping part of the range of two specified taxa.
- Interval biozone include the stata between two specific biostratigraphic surfaces. It can be based on lowest or highest occurrences.
- Lineage biozone are strata containing species representing a specific segment of an evolutionary lineage.
- Assemblage biozones are strata that contain a unique association of three or more taxa.
- Abundance biozone are strata in which the abundance of a particular taxon or group of taxa is significantly greater than in the adjacent part of the section.

4. Index fossils

To be useful in stratigraphic correlation index fossils should be:

- Independent of their environment
- Geographically widespread (provincialism/isolation of species should be avoided as much as possible)
- Rapidly evolving
- Abundant (easy to find in the rock record)
- Easy to preserve (Easier in low-energy, non-oxidized environment)
- Easy to identify (Gehling, 2001)

5. Jeirud Formation

In location of Type section (Jeirud village – north east of Tehran), There are four members (A, B, C and D) and they are with a thickness of 760 meters. Among four members in Jeirud Formation, only member A is pertaining to late Devonian which has been separated, with a clastic unit, with a thickness of 20 meters from other member (B, C and D) in early Carboniferous. Lower boundary of member A is type of parallel unconformity with lilthostraigraphic units (Mila Formation) and is very index but there is no equal opinion about upper boundary.

Amplexograptus, a graptolite index fossil, from the Ordovician near Caney Springs, Tennessee.
(Aserto and et al., (1964))

Aserto and et al., (1964) has known member A, with a thickness of 355 meters in primitive studies, pertaining to late Devonian which has been composed of 140 meters of Plagioclase – bearing Basalt in middle and 55 meters of sand, conglomerate and fossils – bearing limestone at the top. Presence of about 2 meters of grey shale contains Acritarcs, pollen and spores pertaining to last epoch of Devonian (Stronian) in lower boundary of basaltic lavas has been resulted in introducing of Basaltic lavas, in 1960, as boundary between Devonian and Carboniferous and thickness of these strata in upper Devonian decreases from 335 to 140 meters. But Alavi Naiini (1372) and geologist B. R. G. M believe that suggestion of Aserto is more acceptable. Fossils and regional data and lateral elimination of basaltic lavas are emphasis on this belief. Because in areas where there is no Jeirud Formation (member A), Carboniferous has begun with quartzite of member B.

Also, based on advice from national stratigraphy committee in Iran, Jeirud Formation word is only equivalent to member of type section at the present which is representative of upper Devonian rocks in Central and western Alborz B, C, D members in Carboniferous can be compared with Mobarak Formation hence they do not have utility.

It is necessary to mention that one of characteristics of Jeirud Formation is containing of chlore – bearing carbonate apatite (Francolith) which have been observed as granular and or cement particularly in clastic beds and dark shale in lower

bed of formation. In Shemshak Valley, Carat of phosphate – bearing beds is 8-20 % as P2O5. Even though reservoirs of phasohate – bearing beds are economic but concentration process is difficult. Besids of Shamshak valley other section of Jeirud Formation (Firouz kuh, Zakin and Mobaralk Abad) are still symptoms of phosphate which can be compared with phosphate in Central Iran, Zagros and even some of adjacent countries).
(http://www.gsi.ir/newirangeology, 2011)

6. The use of basic biostratigraphy in different sediment types

Basic biostratigraphy is concerned with the recognition of fossils and the relative position of their occurrences in space and time. Various fossil groups can be found in different sedimentary environments. The two main environments are land (terrestrial) and sea (marine).

In terrestrial sediments, spores and pollen are the fossil group to use. Other fossil groups, such as vertebrates or other larger fossils, can only be used if the sediments samples are sufficiently large. In practice, this limits their use to outcrop samples. The limitation on the use of spore/pollen is the amount of oxidation experienced by the sediments after deposition. Oxygen will remove organic material (spores/pollen) from the sediments. Spores can be used in Devonian (approx. 400 myrs) to recent sediments, pollen in Upper Cretaceous (approx. 80 myrs) to recent sediments. Both can be found blown into marine environments in considerable amounts. In marine sediments, calcareous nannofossils are the most useful group for Jurassic (approx. 210 myrs) to recent sediments, since their preparation is cheap and the stratigraphic resolution which can be reached with them is high. Occasionally sediments can be leached of calcareous particles, however, which also removes calcareous nannofossils (for instance in deep sea sediments). Their use as environmental indicators is limited, although some species are known to be associated with warm or cold water masses.

Palynomorphs (dinoflagellates, acritarchs and tasmanites) can be used in Permian (approx. 260 myrs) to recent sediments and in most sediments types (though they tend to be rare in chalky limestone). Since their preparation includes the dissolution of the surrounding sediments, their use is more expensive than nannofossils. The possible stratigraphic resolution can be high. Recently, the use of organic grain size and shape as environmental indicators has been developed and is apparently useful, especially in sub-recent sediments. Foraminifera can be used in all marine sediments which have not be leached of calcareous material, i.e. from shallow marine to middle bathyal. Their stratigraphic resolution can be high and their association with certain depositional environments makes them good environmental (water depth) indicators. Foraminifera can be found in sediments of Carboniferous (approx. 360 myrs)to recent age. Their preparation cost is lower than that of palynomorphs, unless thin-sections have to be used. (http://strats.home.xs4all.nl/biostrat.htm, 2011)

7. Regional geologic setting

Alavi-Naini and Bolourchi (1973) reported that in the area north of Tabriz, sedimentary rocks are presumably of Early to Late Devonian in age (Muli and Ilanqareh Formations) that have transgressed on a Precambrian basement or on Lower Paleozoic sandstones and dolomites of the Lalun and Mila Formations and are in turn overlain by Permian platform dolomites of the Ruteh Formation. The Ilanqareh Formation in the study area conformably overlies the Muli Formation and underlies Jeirud (Devonian) or Ruteh (Permian) Formations.

Paleogeographic investigations indicate that at the time of deposition of Ilanqareh Formation (Devonian age), the northwest Iran was a part of the long and wide northern passive margin of Gondwanaland bordering the Paleo-Tethys Ocean (Sengor, 1990; Beydoun, 1991).

Stump et al., (1995) indicated that the Late Devonian to Early Carboniferous sediments are not uniformly distributed across the Arabian Peninsula due to uplift and erosion associated with the Hercynian Orogeny during the end of Devonian to Carboniferous. Also, regional comparison between Ilanqareh Formation of northwest Iran with the Upper Paleozoic formations in Syria, Iraq, Turkey and Saudi Arabia by Husseini (1991) indicates that during the Paleozoic times, North Africa and Arabia were part of a broad continental shelf margin and, furthermore, these areas were subjected to major intra-continental extension from Late Devonian to possibly Early Carboniferous and the Arabian and adjacent plates were structurally affected by a regional Hercynian tectonic event. (Najafzade et al., 2010)

8. Conclusion

Basic biostratigraphy is concerned with the recognition of fossils and the relative position of their occurrences in space and time. Various fossil groups can be found in different sedimentary environments. The two main environments are land (terrestrial) and sea (marine). Biostratigraphy is the branch of stratigraphy which focuses on correlating and assigning relative ages of rock strata by using the fossil assemblages contained within them. Usually the aim is correlation, demonstrating that a particular horizon in one geological section represents the same

period of time as another horizon at some other section.

Based on advice from national stratigraphy committee in Iran, Jeirud Formation word is only equivalent to member of type section at the present which is representative of upper Devonian rocks in Central and western Alborz B, C, D members in Carboniferous can be compared with Mobarak Formation hence they do not have utility. It is necessary to mention that one of characteristics of Jeirud Formation is containing of chlore – bearing carbonate apatite (Francolith) which have been observed as granular and or cement particularly in clastic beds and dark shale in lower bed of formation.

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The Study of Some Cognitive and No cognitive Variables as Predictors of School Success and Unsuccessful in the First Year Male High School Students of Ramhormoz

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Abstract: The purpose of the present study was to investigate intelligence, emotional intelligence, academic self-concept, locus of control and social-economic status as the predictors of academic success and failure of first year high school male students in Ramhormoz. Thus, from all successful students and unsuccessful students 160 persons selected randomly. The materials used in this research are as follows: Petrides & Furnham Emotional Intelligence Inventory, Delavar Academic Self-concept Inventory, Rotter Locus of Control Inventory and a researcher-made Social-Economic Status Inventory. Also Raven's Standard Progressive Matrices score were used from the students personal files. In order to analyze the data both discriminate analysis and descriptive statistics were used. Findings revealed that except component social skills concerned to variable emotional intelligence that was not a predictor of academic success and failure, other variables confirmed the hypotheses of this study and revealed that success or failure male students can be predicted from the variables intelligence, emotional intelligence, academic self-concept, locus of control and social-economic status. The obtained results and the model presented in this study are discussed in detail.

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Keywords: Intelligence; emotional intelligence; academic; self-concept; locus of control and social–economic status

1. Introduction

Academic failure is one of the important concerns of families and education experts. Every year a great number of students in different countries encounter academic failure. This phenomenon in addition to the great economical damages has adverse effect on students and makes the families concerned. Therefore, this case should be analyzed carefully as one of the important social and educations problems and there are different factors on educational success and failure of students that determination of the most important factors is effective to help teachers and parent –teacher for educational planning, assessment and educational success of students. Educational success and failure are discussed a lot. The role of some factors on educational success and failure or the share of each factor is always of interesting fields for education researchers and psychologists. One of these influential factors is IQ and mental abilities (Biabangard, 1380). The investigations done in this field indicate the reality that successful students necessarily are not smart and unsuccessful students are not surly students with low IQ, but a great number of students with average or high IQ fail in their lessons and the students with lower average IQ can achieve as much educational goals (Nazari,1377). Also, other researches (as Carlson & Johnson, 1988; Shofild &Ashman; quoting Glover & Bruning, 1990; Translation of Kharazi, 1383) show that intelligent students regarding working memory, perception and access to long-term memory and information processing scale are better than normal students.

Today IQ is not very important as before and it is said that any person progress in life with his/her own characteristics ladder and between two students with similar IQ, the one who works hard is more successful (Biabangard, 1380). Goleman (1995) believes that IQ can not ensure success beyond the school and characteristics factors including, motivation, creativity, inclination to do hard works and EQ play important role in achieving success. Therefore, considering the limitation of prediction ability and determination of education progress variance of recognition variables, the researchers emphasized more on the necessity of considering extensive educational success predictions. Here the role of social and excitement competencies in education success is one of the modern fields of research.

Theoretical origin of these researches is the concept of EQ proposed by Salovey & Mayer (1990, quoting Shalchi, 1385). In addition to IQ, another factor in the education success or failure is EQ. In this case, psychologists believe that to educated people to have strong self-concept and be successful in education, it is necessary to take attention to both IQ and EQ (Nori Emamzadehi, 1383).

Academic self-concept is also the most important issue in success or failure of students in education. The way the student see him/herself has great influence on education performance and the positive and negative self-concept of people is formed and established by the feedback receiving from others and they are influenced by the results of their performance. Seligman (1974, quoting Nazari, 1377) states that unsuccessful students have negative beliefs about their abilities and probably at first there had little effort, and due to the fact that the effort was vane, they couldn't achieve success. So, they concluded that they are not able to achieve success. Also, about academic self-concept Seif (1384) believes that a students who had successful experiences in the school has great interest in learning different textbooks or in general in the school itself. So, he/she can have a positive concept about himself. In addition, other researches (Byrne, 1984; Bridgman, 1975, quoting Karimzadeh, 1384) have reported positive and high correlation coefficient (0.40 to 0.60) between self-concept and academic progress. Also, academic self-concept can be influenced by internal and external evidences of people, Because, today locution of control is the important issue in academic success and failure of students. Seif(1384) also states that the way (perception and interpretation) people think about success and failure may influence their academic self-concept and changing documents of learners show them that achieving success and learning control is as much controlled by them and this is not luck that brings success for them.

Researches show that the people with internal control in comparison with the people with external control in verbal tests are very active, explorer, inclined to progress, powerful and independent, responsible, sociable, patient and strong thinkers (For example see Karbalayi Harfte, 1383). Of other important issues influencing academic success and failure is their social-economical status. Generally, environmental deprivations and the lack of mental and social incentives are important factors of student's academic success. A considerable percent of students who fail in education, are forced to go to exceptional schools and they are regarded as << culturally retarded>> people and students who belong to families with low social and economical status, are faced at the same time with economical and cultural poverty and are less successful in education (Biabangard, 1380). The main question of the current paper is that "Can IQ, EQ, academic self-concept, locus of control, social-economical status can predict academic success or failure?

The subject of the research is analyzed also in abroad. Stein mayr, Ziegler & Trauble (2009) in a research on IQ, for grade 11 and 12 students in Germany found that the paying attention and supporting students influence IQ and indeed the educational performance and both are predictors of school educational performance.

Grayson (1999) in a research stated that IQ is the best predictor of education success, because in this research the average IQ of 15% of unsuccessful students was under 110. Also, Alexander (1985) in a study analyzed the different opinions of smart and normal students at 12 to 17 age and indicated that smart students know their superiority due to internal motivation factors such as effort and feedback. He concluded that the reason of smart student's success is the internality of their locus of control.

Marjorie, Parker, Wiener, Watters, Wood, & Oke (2009) in a research on 192 girl and boy students from Canada showed that IQ, EQ and social support predict academic success. In addition, the research of Parker & Marjorie (2006) in Canada indicated that successful group who were more stable in education in comparison with unsuccessful group, have more interactions, interpersonal adaptation and ability to control stress. Therefore, he concluded that EQ is a good predictor for academic success ($p < 0.001$).

Suntonrapot (2009) in research on 820 nine-grade students in Tiland by multiple correlation, MANOVA repeated measures, showed that there is correlation between academic and non-academic self-concept and academic progress. This model is compatible with experimental data.

Pullmann & Allik (2008) in a national research on 4572 Estonian students found that general and education self-esteem are strong predictors of academic progress in school and academic failure cause defensive mechanisms such as pessimism toward educational abilities. Also, Wilkins (2003, cited in Kashkulinejade kuhi, 1384) found that there is correlation between self-concept and academic progress such as progress in mathematics.

Hansford & Hattie (1982, cited in Javir and Sanchez, 2005) in his research identified the relationship between academic self-concept and academic progress about 0.77 to 0.96. Uguak, Elias, Uli & Suandi (2007) in a research on 210 foreign students living in Malaysia found that locus of control has a meaningful relationship (0.52) with academic progress. Hoover (2003) also in a study on southern Carolina students indicated that locus of control predicts 49 to 53% variance of academic success. Donald & Wessler (1994, cited in Soleimani nejad, 1381) in some researches expressed that people who attribute their successes to external factors, are more stressed and this avoids success

expectancy for them and this person will be doubtful of his abilities to get successful.

O'Connor (2008) in a study on the social-economics status revealed that the parents education level, cultural, social and interpersonal deprivations are related to academic failure. Also, Pain & Bidel (1998, cited in Caldas, 2000) carried out a research in Louisiana of America and indicated that social-economical status predict variance of 45.5% and poor families with two children in comparison with poor families with only child have more negative influence on academic progress.

Regarding the current research subject, some researches' are done in our country. Naghibi (1375) in a research on third guidance school boys in Ahvaz revealed that there is positive and meaningful relationship between IQ and students academic performance in P=0.001 level (r=0.159). Alizadeh Darbandi (1385) in a pilot research on high school girl students in Mashhad showed that excitement skills education including adaptability, overcome stress meaningfully promote EQ that improve mental health and success in life and education. Also, the results of Dehshiri (1385) research on 153 girl and boy students in high school revealed that there is a meaningful correlation between EQ and academic progress. Regarding academic self-concept Karimzadeh (1384) in a research about the relationship between general and academic self-concept with the academic progress among girl students of second of high school majoring mathematics and humanity in region 6 of Tehran showed that two groups have meaningful difference in p <0.0001in terms of general and academic self-concept and the more positive the academic self-concept, the more academic progress. Tahernejad (1383) in a research on 390 students of Shahid Beheshti University revealed that there is a meaningful relationship between locus of control and academic progress. Also Shahraray and Soleimani nejad (1380) in a research on 150 students of third of high school majoring in mathematics in Tehran showed that there is negative relationship between external control source with academic progress from luck aspect and internal control source has positive relationship with academic progress.

Lavasani and Dorani (1383) in a research titled " The review of the relationship between personal and family characteristics with academic progress of psychology and educational science students of Tehran University by regression analysis concluded that education, fathers job, family income, living condition and diploma average has a meaningful relationship with academic progress of students (p <0.01)

By reviewing the results of the previous researches, we can find that the major problem of these researches is that they have studied limited variables separately while to understand the prediction of academic success or failure it is necessary that in a uniform research IQ, EQ, academic self-concept, locus of control and social – economic status should be analyzed.

Considering the role that related literature expresses for the current research variables, the general goal of this research is that by IQ, EQ, academic self-concept, locus of control and social-economic status as prediction variables, the success or failure of first of high school are predicted.

2. Methodology

Statistical population of this research is including all successful boy students (passed) and unsuccessful students (failed) of first high school in Ramhormoz town studying in 1386-87. After making a list of the names of all passed and failed students, 225 successful students (passed) and the same number of unsuccessful students (failed) was selected as simple random and questionnaires were given to them. After putting aside incomplete questioners and the people who did not fill the questionnaire, the data of 160 successful students (passed), and 160 unsuccessful students (failed), were analyzed. It is worth to mention that successful students (passed) were randomly selected from the same school that unsuccessful students (failed) were studying.

Research instruments

1) Ravens intelligence test

Raven's Progressive Matrices were built in England by Raven in 1954 and it aimed to measure general intelligence. This test required finding reasonable relations in abstract matters and it was recognized as the best criterion for general intelligence factor from England psychologists. (Anastasi, 1976, cited in Sepahvandi, 1385). This test is consisting of 60 matrices in which some part is eliminated and the subject should find the deleted section among 6 or 8 different choices. The test cases are divided into 6 groups and each group is including 12 cases in which difficulty level is increasing gradually. In the first cases, only true recognition is important but in difficult cases of scale, the design change and other reasonable relations are discussed (Sepahvandi, 1385). Raven's Progressive Matrices is analyzed from reliability and validity in different forms. From re-test reliability aspect, correlation coefficients range is from 0.85 to 0.96 and from split-half reliability the range is 0.88 to 0.97 (Shekarkan and Haghighi, 1373, cited in Sepahvandi, 1385). The power of progressive matrices in the prediction of academic success is reported as between 0.35 to 0.65 (Baraheni, 1364,

cited in Falahati, 1378). Sepahvandi (1385) calculated the reliability of this test by split half method as 0.89. From validity aspects, matrices are correlated with the student's scores. For the latter correlation the range is reported from 0.30 to 0.80 (Sepahvandi, 1385)

2) EQ questionnaire of petrides and furnham

In this research EQ scale of petrides and furnham (2000, cited in Ali Akbar Dehkordi, 1386)is used to measure EQ

This scale is made with the change of shot scale (1998, cited in Ali Akbar Dehkordi, 1386) and by using factors analysis method on the analysis of this scale. Petrides and furnham 2000, cited in Ali Akbar Dehkordi, 1386) believed that by modifying this scale, they could identify people who need guidance and counseling. In addition, this questionnaire comprises 30 items, measuring the characteristics of EQ in four components including optimism, emotion perception (self and others), emotion management and social skills. In half of the questions in petrides and furnham test, scoring method is reversed (Guesstlow & Guesstlow, 2003, cited in AliAkbar Dehkordi, 1386).

Petrides and furnham (2000, cited in Ali Akbar Dehkordi, 1376) reported reliability of EQ scale and sub scales with Cronbach's α 0.76 to 0.86. In addition, AliAkbar Dehkordi (1386) reported the reliability coefficient of all the scale by Cronbach's α as 0.77 and for sub scales as 0.70, 0.76, 0.77 and 0.75. In the current research the reliability of the test by Cronbach's α and split-half coefficients for all the questionnaire is obtained respectively as 0.67 and 0.65 and for sub scales it was obtained respectively 0.39, 0.62, 0.34 to 0.55. Petrides and furnham (2000, cited in Ali Akbar Dehkordi, 1376) reported its high internal sensitivity to determine the validity of this scale. Also, AliAkbar Dehkordi (1376) reported the validity of petrides and furnham EQ by correlating it with shot EQ test (1998, cited in by Shahbazi, 1384) for all employed women and men in Ahvaz as 0.40 for all the validity coefficient scale and micro scales respectively 0.39, 33, 0.37 and 0.35. All of these values were meaningful at $p < 0.001$. In the current research petrides and furnham EQ questionnaire was calculated by correlating its scores with Shot (1998) EQ scores that for all the questionnaire correlation ratio of 0.40 and for micro scales, correlation ratio of 0.14 to 0.52 was obtained that are meaningful at $p<0.05$.

3) Delavar academic self-concept scale

This scale is provided by Ali Delavar (1373, cited in Ebrahimi Ghavam, 1377), with 40 items. Ebrahimi Ghavam (1377) used this scale in a research titled as" The effective factors on academic failure of Allame Tabatabai University students". He stated its reliability by Cronbach's α 0.78. In the current research its reliability coefficient was calculated by Cronbach's α coefficients and split-half as respectively 0.84 and 0.69.

According to the experts in this field, the test is suitable from content validity (Ebrahimi Ghavam, 1377). In the current research to determine Delavar academic self-concept, criterion validity method is used (correlating the total score of questionnaire with 5 questions made for academic self-concept). Here the given coefficients was 0.49 and it was meaningful at $p<0.001$.

4) Rutter locus of control

This scale was provided by Rutter (1966) and compromise 29 items and each item is consisting of two sentences. Scoring method of this scale is as zero and one. So, for reversed questions to section "b", each question is score 1and for section "a" zero is given to each question and in the other questions score 1 is given to questions "a" and zero is given to questions "b".

In the main sample, high score is considered as external direction. In this research scoring method is the main sample method and subjects who get 9 or more, have external control source and people who get less score, have internal control source.

Hirsh & Skib (1967, cited in by Allame, 1381) reported the reliability coefficients of Rutter scale by test-retest method in different samples in time duration of 2 months as 0.48 to 0.84. Saburi Moghadam (1372 cited in Yahyazadeh, 1384) by split-half method, obtain the reliability of this scale about 0.81. Validity of Rutter locus of control scale is reported by correlating it with Nowicki & Strickland test by Nowicki & Strickland (1973, cited in by Allame, 1381) on a sample of 76students as 0.61. In addition, Movafagh (1375, cited in by Yahyazadeh, 1384) showed the validity of this scale by using concurrent criterion validity; it means that Nowicki & Strickland internal and external control source scale is 0.39 as a criterion.

To analyze Social-economical status of students, researcher-built questionnaire was used. This questionnaire is composed of 10 items about the personal, family and economical status of students and each question has 3 choices.

3. Results

Table 1 shows the average and standard deviance of successful or unsuccessful students in research prediction variables.

Table 1- Average and standard deviance scores of successful or unsuccessful students in research prediction variables

Predictive variables	Successful students		Unsuccessful students	
	Average	Deviation	Average	Deviation
Intelligence	44.35	8.47	33.39	10.55
Emotional intelligence	139.45	16.69	132.97	17.86
Optimism	36.79	6.64	34.46	7.32
Own and other senses understanding	45.69	7.4	43.91	8.1
Evaluation and control of emotions	30.81	6.90	28.97	7.09
Social skills	26.15	5.65	25.63	5.62
Academic self-concept	116.12	11.76	104.61	11.81
Locus of control	8.24	3.25	10.28	2.67
Socioeconomic status	17.08	4.49	14.75	2.84

The content of table 1 shows that average and standard deviance scores of successful group in intelligence variable is respectively 44.35 and 8.47 and in unsuccessful group is respectively 132.97 and 17.86. The average and standard deviance scores of successful group in pessimism variable as the component of EQ are respectively 36.79 and 6.64 and in unsuccessful group are 34.46 and 7.32.

The average and standard deviance scores of successful group emotion perception (self and others) variable as the component of EQ are respectively 45.69 and 7.4 and in unsuccessful group are 43.91 and 8.1.

The average and standard deviance scores of successful group in emotions control variable as the component of EQ are respectively 30.81 and 6.90 and in unsuccessful group are 28.97 and 7.09.

The average and standard deviance scores of successful group in social skills variable as the component of EQ are respectively 26.15 and 5.65 and in unsuccessful group are 25.63 and 5.62.

The average and standard deviance scores of successful group in academic self-concept variable as the component of EQ are respectively 116.12 and 11.76 and in unsuccessful group are 10.28 and 2.67.

The average and standard deviance scores of successful group in social-economic statues variable as the component of EQ are respectively 17.08 and 4.49 and in unsuccessful group are 14.75 and 2.84.

Research hypothesis indicates that linear combination of IQ, EQ, academic self-concept, locus of control, social-economic status predict the academic success and failure of boy students of first of high school in Ramhormoz. The results of table 2 directly and results of table 3and 4 indirectly is related to research hypothesis.

Table 2- The summary of Canonical discriminant function results by Enter method (5 predictive variables) and stepwise method (5 predictive variables)

Important information related to discriminant function	Co-occurrence discriminant discussion	Stage to stage discriminant discussion
Function quantity	1	1
Specific value	0.669	0.669
Variance (%)	100	100
Compression (%)	100	100
canonical correlation	0.633	0.633
Etta square	0.400	0.400
wilks lambda	0.599	0.599
K Square	161.582	161.582
Freedom level	5	5
discriminant function significance	0.001	0.001
Data central for successful group	0.815	0.815
Data central for unsuccessful group	-0.815	-0.815
Group membership prediction	79%	79%
Kappa coefficient	0.588	0.588
Significance of kappa coefficient	0.001	0.001

As it is shown in table 2 (both in discriminate analysis by Enter method in which 5 variables entered into analysis and in stepwise discriminate analysis in which after entering all the variables, each 5 variables remain in the analysis and due to this the results of analysis by both methods are the same), considering the amount of small Lambda and the amount of large Chi- square and meaningful level $P<0.001$, the obtained discriminant function has suitable discrimination power to determine the dependent variable (group in successful and unsuccessful levels). Therefore, the hypothesis is true. To understand the first column (important information about discriminant function) of table 2, explanations of number 1 to 10 are shown in the followings. Besides, the results of 5 variables analyzed with stepwise method are shown in table 3 and 4.

Table 3- The summary of stepwise discriminate analysis results with wilks lambda of 5 predictor variables

Step	Imported	Variables quantity	wilks lambda	1st Freedom level	2nd Freedom level	3rd Freedom level	Fixed F			
							Statistics	1st Freedom level	2nd Freedom level	Significance
1	Intelligence	1	0.752	1	1	318	105.002	1	318	0.001
2	Academic self-concept	2	0.634	2	1	318	91.318	2	317	0.001
3	Socioeconomic status	3	0.621	3	1	318	64.388	3	316	0.001
4	Emotional intelligence	4	0.611	4	1	318	50.131	4	315	0.001
5	Locus of control	5	0.599	5	1	318	42.005	5	314	0.001

Table 4- The standard coefficients table, non-standard, structural coefficients and classification coefficients of discriminant function by Enter and stepwise method

Predictors		Function									
		Enter method					Stepwise method				
Code	Variable	Standard coefficients of discriminant function	Non-Standard coefficients of discriminant function	Constructional coefficient	Classification coefficients of discriminant function		Standard coefficient of discriminant function	Non-Standard coefficients of discriminant function	Constructional coefficient	Classification coefficients of discriminant function	
					Successful group	Unsuccessful group				Successful group	Unsuccessful group
X_1	*Intelligence*	0.672	0.070	*0.703	0.497	0.383	0.672	0.070	*0.703	0.497	0.383
X_2	Emotional intelligence	-0.255	-0.105	0.230	0.246	0.270	-0.255	-0.015	0.230	0.246	0.270
X_3	Academic self-concept	0.643	0.055	0.599	0.793	0.704	0.643	0.055	0.599	0.793	0.704
X_4	Locus of control	-0.230	-0.077	-0.419	2.216	2.342	-0.230	-0.077	-0.419	2.216	2.342
X_5	Socioeconomic Status	0.278	0.074	0.380	0.602	0.481	0.278	0.074	0.380	0.602	0.481
-	Constant number	-	-7.195	-	-89.204	-77.473	-	-7.195	-	-89.204	-77.473

*The highest absolute correlation between each variable and discriminate function

1. The number of functions: Number 1 indicates the only discriminant obtained by two levels of criterion variable.
2. Eighen value: By considering table 2, eighen value is just discriminant function 0.669(Enter method) and 0.669 (stepwise method). Generally, the more the amount of eighen value in a function, the more discriminant is the function (Khojastemehr, 1384)
3. Variance percent: It is clear that as there is a discriminant function, the variance is 100%. In some cases with many discriminant functions, the more valuable function is the one with more variance percent.
4. Canonical correlation and its square (Eta squared): According to the information in table 2, this value in discriminant function by Enter method is 0.633 and by stepwise analysis is 0.633. Eta squared for discriminant analysis by Enter method is 0.40 and by stepwise method is 0.40. Therefore, 40% of diffraction is 5 predictor variables by Enter method and 40% diffraction of 5 predictor variables by stepwise method for the only discriminant function is determined by the difference between two successful and unsuccessful groups.
5. Wilks lambda: As it is shown in table 2, this amount for discriminant function by Enter method is 0.599 and for discriminant function by stepwise method is 0.599 that both values are meaningful at $p<0.001$, so the research hypothesis is proved.
6. Chi- square: As it is shown in table 2, this amount for discriminant function by Enter method is 161.582 and for discriminant function by stepwise method is 161.582. According to the value in meaningful row of discriminant function, Chi- square is meaningful at $p <0.001$. Therefore, the research hypothesis is proved. So, we can say that the given discriminant function as meaningfully is having high discrimination amount for two levels of criterion variables. Considering the above explanation in number 5 and 6 the hypothesis for Enter method is $p <0.001$ and $X2(5, N=320)=161.582$ and $=0.599$(Wilks lambda) and for stepwise method $p<0.001$, $X2(5, N=320)=161.582$ and $=0.599$ (Wilks lambda).
7. Score Centroid: As it is shown in table 2, score Centroid for the only discriminant function by Enter method for successful group is 0.815, in unsuccessful group is 0.815, and by stepwise method is 0.815 for successful group and 0.815 for unsuccessful group. This means that cutting point of academic success and failure in students for discriminant function is zero and the given discriminant function is a suitable discrimination function to separate the successful and unsuccessful group. Thus, if the scores of one group is being put in discriminant equation and the score is positive, it is predicted that the group is successful and if it is negative, the group is unsuccessful.
8. The prediction of group membership: The given discriminant function by Enter method (5 predictor variables) generally 79% of groups and with stepwise method (5 predictor variables) 795 of groups are well classified.
9. Kappa coefficient: This coefficient shows the modified precision of prediction (Khojastemehr, 1384). In explanation No. 8 it was said that the given discriminants function prediction power is 795 by Enter method. As it is shown in table 2, the modified precision of this prediction is 0.588. The modified precision of prediction power of discriminant function by stepwise method is 0.588.
10. Meaningfulness of Kappa coefficient: Kappa coefficient for the given discriminant function by Enter and stepwise method is meaningful at $P<0.001$. Thus, it can be said that prediction power of the above discriminant function is meaningful.

Stepwise discriminate analysis of academic success and failure of students was done on 5 predictor variables (Intelligence, EQ, academic self-concept, locus of control and social – economical status) and the results are shown in table 3. According to the information of this table, after presenting 5 variables, each 5 variables remained. In the first stage, IQ in the second stage academic self-concept, in the third stage, social-economic status, in the fourth stage, EQ and in the fifth stage, locus of control came into analysis in which F for 5 variables at $P<0.001$ is meaningful.

Standard coefficients, non-standard, structural and classification of predictor variables of discriminant function are shown in table 4. As it is clear from the information in table 4, for the only discriminant function with two analysis, four types of standard coefficients, non-standard, structural and the classification of discriminant function are shown. As it is shown in standard coefficient column of table 4, by Enter method, IQ variable is the highest and again

in stepwise method, IQ variable is the highest variable. By non-standard coefficients shown in table 4, discriminant function equation is obtained. Thus, by putting the scores of each group in the function, the predicted score is obtained. Considering the scores Centroid of successful and unsuccessful groups in table 2, if the discriminant score is positive, it is predicted that the group is successful and if it is negative, it is predicted that the group is unsuccessful. Regarding the column of non-standard coefficients and constant number of discriminant function by Enter method the following predictor equation was obtained.

$)X_5 (0.074 + X_4) (0.077) - X_3 (0.055+ X_2) (0.015 -$

$X_1) (.0.070 + 7.195= - y' =D$

Regarding the column of non-standard coefficients and constant number of discriminant function by stepwise method the following predictor equation was obtained.

$)X_5 (0.074 + X_4) (0.077) - X_3 (0.055+ X_2) (0.015 -$

$X_1) (.0.070 + 7.195= - y' =D$

It is worth to mention that y' or D is the discriminant score or predictor score of each pair. AS it is shown in table 4, for both Enter method analysis and stepwise method, classification coefficients are indicated. According to the column of the classification coefficients of discriminant function by Enter method of successful group in comparison with the unsuccessful group IQ,, academic self-concept and social-economic status show higher scores and EQ and locus of control have lower scores. The same condition is true about classification coefficients by stepwise method for each 5 variables in the analysis. In table 4 respectively structural coefficients of Enter and stepwise method, the biggest absolute correlation is between IQ and the only discriminant function. As it is shown in table 4, IQ, academic self-concept, locus of control, social-economic status and EQ respectively have the highest correlation with the only discriminant function with 5 predictor variables by Enter method. As it is shown in table 4 IQ , academic self-concept, locus of control, social-economic status and EQ variables have the highest correlation with the only discriminant function with 5 predictor variables by stepwise method. It is worth to mention that discriminant function may be named to determine the variable that can have the highest relation with it. Considering the information in table 4, the only discriminant function has the highest correlation with IQ variable. So, we can name the only discriminant function as IQ. Considering the classification coefficient of discriminant function and concurrent values and with stepwise method, we can create two discriminant equations and by putting the scores of each pair in two equations, two discriminant scores are obtained. If the given score is close to the scores Centroid of successful group, it is predicted that it is a successful group and if the score is close to the scores Centroid of unsuccessful group, it is predicted that it is an unsuccessful group.

4. Discussion and Conclusion

The results of the current research was with the prediction purpose of academic success and failure by IQ , EQ , academic self-concept, locus of control and social –economic status. The results as was expected proved the purpose of the research. Regarding IQ, the results of the current research are compatible with the results of stein Mayer, Ziegler & Trauble (2009), Lasiter and Baradus (2006), Riedel and Lansbury (2004), Thelma (1998), Karbalayi Harfte (1383), Khalatbari (1376) and Naghibi (1375)

Grison (1999) in a research reported that IQ is the best predictor of academic success, because the average IQ of 15% of unsuccessful students was under 110. Anderson (1992) believed that the intelligence difference of people is due to the different dealing of the initial processing and thinking that itself leads into knowledge. Thus, there is difference between people with the initial processing speed of information. In addition, Shofild &Ashman (cited in Glover and Bruning, 1990, cited in Kharazi, 1383) reported that intelligent students in terms of information processing are better than high average students and high average students are better than low average students. They came to the conclusion that the students with higher IQ in comparison with the students with lower IQ, have better academic performance. On the other hand, according to Graham (1986, cited in Shahni Yiylagh, 1375) stating about IQ, IQ with the high leaning ability, positive attitude to the problems and solving them, rapid conclusion, creativity and concentration lead into academic success. So, we can say that a percent of academic failure is due to the students mental inability.

Regarding EQ, the result of the current research is compatible with the results of Marjorie, parker, Wiener, Waters, Wood& oke (2009), Abraham (2006), petrides, Frederickson and furnham (2004), Shalchi (1385) and Dehshiri (1385).

Congelosi & Peterson (1998) stated in their research that students who fail in the school have weak communication skills. But students with high EQ, have less deviant behavior, escape from school and expulsion from school. Also, Pamplin (2005) in his research showed that the students with high EQ, have higher self-efficiency and self confidence and they can better cope with problems. In addition, Shot and Malof (2002) in a research on freshman students found that the students, who participated in the

instructional course of EQ, have higher academic success and social adaptability in comparison with the student who didn't participate in this course.

Regarding academic self-concept, the results of the current research is compatible with the results of pullmann & Allik (2008), Avad (2007), Javir and Sanchez (2005), Karimzadeh (1384) and Nasr Esfahan (1382)

The researches showed that teenagers who value themselves are successful in dealing with others and are more stable facing difficulties. If these people know consider themselves weak, give up very soon (Marsh and young, 1999 cited in Karimzadeh, 1384). Also, Seligman (1974, cited in Biabangard, 1380) believe that unsuccessful students have negative feelings about their abilities. Probably these students work a little, but due to the vain efforts, they can't achieve success and they found that they are not able to achieve success. This feeling leads into the lack of effort and if they insist on their belief, it is not expected that they learn anything.

Regarding locus of control, the results of the current research is compatible with the results of Uguak, Elias, Uli & Suandi (2007), Hoover (2003), Karen (2002), Tahernejad (1383), Shararay, and Soleimani nejad (1380). The positive outcome of behavior that is attributed to the internal reasons such as ability and effort, create pride and self-esteem feeling in a person. But the success attributed to external factors such as luck or others help doesn't create pride and self-esteem feeling in a person. However the failure attributed to less ability, is leading more to embarrassment in comparison with the failure attributed to difficult duty or bad luck (Weiner, 1977, cited in Seif, 1384). Also, Flowers, Milner and Moor (2003) in their research found that the students with internal locus of control in comparison with the students with external locus of control have high academic expectancies. In addition, Donald & Wessler (1994, cited in Soleimani nejad, 1381) in some researches expressed that people who attribute their success to external factors, get more stressed and this many avoid their expectancy from success in future. So, the person doubts about his abilities to achieve success.

Regarding social-economic status, the results of this research are compatible with the results of Cutter and Guma (2006), Ingwiler (2005), Caldas (2000), Zaki (1384), Lavasani, and Dorani (1383).

The parents of low social and economical status emphasize more on respect toward authority, but the average status, insist more on curiosity growth, responsibility, internal control and working due to long-term goals and being sensitive to in communication with others (Tahurian, 1373, cited in Fathi, 1385). Also, the parents in high social-economic status have higher dreams and motivations in comparison with the low social-economic statues families. So, they expect that their students be successful in their studies and they try to support and encourage them and force others also to help their children (Zare, 1374, cited in Biabangard, 1380). In addition, O'Connor (2008) in a study on the social-economics status revealed that the parents education level, cultural, social and interpersonal deprivations are related to academic failure. Also, Pain & Bidel (1998, cited in Caldas, 2000) in a research indicated that social-economical status predict variance of 45.5% and poor families with two children in comparison with poor families with only child have more negative influence on academic progress. To make the results of this hypothesis clearer we can say beside the above explanations that some percent of academic failure is due to less attention in childhood, growing up in an environment without incentive or motivation and also mental inability and the lack of ability to learn. Also, high EQ with increasing positive mood changes for adaptive interaction in person, thinking organization and memory content, increasing the emotions recognition to cope with stressful situations and the management of disasters by emotions regulation all lead into the increase in academic success and its effective activity. On the other hand, the positive self-concept increase capability, high-mindness and ensuring thinking in the person, so, the person tries to do the works by selecting the real goals and doesn't lose the successful behavior opportunities. Also high internal locus of control make the person thinks that he has positive intervention on his destiny and their life events are the result of precise planning and continuous efforts. Therefore, they are not passive toward their life events and accept the responsibility of their behavior and deeds and try to achieve success. Also the low social-economic status and the lack of thinking in families and parents cause that they couldn't have good attitudes and emotional and logical relationship with themselves and their children to help them in their lessons or provide them with good educational instruments. In return, in high social-economic status, children do not have such problems.

As it was said, the research model was proved. In other words, by IQ , EQ , academic self-concept, locus of control and social-economic status variables we can predict academic success or failure and based on this model, a model can be provide to prevent academic failure or improving deficits in academic performance. As it is clear from the results of the research, intelligence in the current model has the main role and in this case, there is a reciprocal relationship between intelligence level and the

quality of academic output. Thus, to help students for improving the academic quality and achieving success, we should not ignore the importance of increasing their intelligence. By having an effective model by which we can predict intelligence assessments, EQ, academic self-concept, locus of control and Social-economic status before academic performance, the effect of intervention and problem-solving increase.

The limitations of the current research are as the followings: 1- being cautious about generalizing the results of the current research to girl students and other grades and other cities or provinces in the country, 2- less certainty of the current research results in comparison with experimental and quasi-experimental methods due to the correlation nature of information and not showing causative relations between variables and 3- The studied variables are not comprehensive representation of all the variables and approaches about academic success and failure.

It is recommended to analyze other personal, family, social and institutional factors effective in academic success and failures in future studies and do the researches similar to the current research about girl students and other provinces of the country. Also, as the strongest predictor of academic success and failure was intelligence, it is recommended that educational researchers and experts use the results of this research in health-instructional interventions.

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Evaluation of Imprisonment, Good or Bad?

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Abstract: Nowadays, imprisonment is exerted for the delinquent who commit crimes and do not observe the social, human and moral values throughout the world. It can, without exaggeration, be said that there is no country in the world that has not anticipated the imprisonment in its statutory and does not have prisons and prison wardens because the penal role of the prison is undeniable, and it is impossible for a society to survive and continue without the existence of the prison. Since the late middle ages and renaissance, prison has been used as a punishment. Consequently its usage has reached its peak and has had a very long record in the history. Its history is really deplorable because the prison has been considered as a place so exert a series of punishing measures and/or as a detention house for the accused. But it is different from the organized form of these punishments in the current era because the prisons had not had favorable conditions in the past, and the prisoners were kept in dungeons, castle undergrounds and in the dark and humid places. Thus, many prisoners died of the unfavorable environment of the prisons during their imprisonment.
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Keywords: Imprisonment, Law, Punishment

1. Introduction

The historical surveys indicate that imprisonment, as a social reaction to punish the delinquent, reform, treat and readjust him/her socially is more suitable than other types of punishment such as burring alive, amputation, whip, death, etc. and is considered as a reduction in punishment. Because the aftermath of such punishment as death, amputation, etc. and more merciless and terrible than imprisonment. In fact, imprisonment as a main punishment is relatively new and current punishment compound to the above-mentioned corporal punishment. The punishment of imprisonment in its today form and approach has entered the modern criminal law, so is a new punishment. In the Islamic Republic of Iran, too, it has been used wildly since 1925 upon the approved of Public Punishment Art. The extent of its usage is such that this imprisonment has been predicted for around four hundred offences in the criminal era of the Islamic Republic of Iran(2).

Considering the philosophy of the imprisonment enactment, which is to punish and return the criminals, these days it can be seen that the prisons face an inflation of the penal population. Judges in many countries, specially in Iran issue the order of imprisonment for many offences. Government do not allot enough budget to the prison institution. The prison wardens are not given enough training and do not attend to this penal role. Children are imprison together with adult prisoners and are sometimes kept in the same cell. Usually mentally disordered patients are sent to prison instead of lunatic asylums. And the number of the prisoners increases every day. Unfortunately, we can see that the prisoners go back to prison again as a result of committing offences after their releases from prison like other countries, in Iran too, this problem occurs to the prison and prisoner. Has the imprisonment tailed? Has the life of prisoner come to an end? Do we have to seek for replacements for imprisonment? These are attempt to investigate the role, studding and function of imprisonment discuss proponents and opponents of imprisonment and provide a precise evaluation of this punishment.

2. Proponents of imprisonment

As pointed out earlier, imprisonment has been accepted as a principal punishment throughout the world and institutionalized in Iran, too, and turned out to be a social fact and phenomena. And the authorities (the judiciary, organizations of educational, security measures and prisons) make special attempts to institutionalized the prison measured. This indicates that imprisonment is considered as one of the important punishments because such reasons as (preventiveness, exemplariness, reformative- ness and remediability in case of judicial mistake and/ or the defendant reformations) creating order and protecting the

society against the delinquent account for these punishments(3).

The defects like inappropriate conditions of prisons in terms of physics, failure to allocate suitable budget and/ or failure to train the prison staff and the increase in the penal population of prisoners, etc. can be removed by means of allocation of budget, employment of staff and training of the orison wardens. Thus it impossible to ignore and disregard the undeniable role of the imprisonment since prison has advantages as follows:

1- Exertion of Punishments

Based on the teachings of the Islamic commandments, freedom and option is one of the primary rights of the human being. Therefore, the more lack of this freedom is unfavorable to the human being. When a criminal is put in prison, he/she is, in fact, punished hard, so the most significant of this punishment is to punish the criminal.

2- Protection of Society and Criminal

A delinquent who violates the statuary of a country treads the individuals and societal rights in a way and disturb the social system. If this person is not reacted to, the society suffers and gets chaotic. It may also lead the society to an abyss. Thus, this person should be kept away from the society until perfect reformation, and it becomes possible only through imprisonment. In addition, the imprisonment is advantageous because the criminal who has committed a crime and is under arrest by the family of and/ or the victim himself can be protected because the experience shows that the anger and pain of the victim and his family abates in the passage of time when the criminal is in prison, and it is also possible that he is forgiven in the future. Additionally, imprisonment is superior to the punishments. Firstly, compared to death that does not have the possibility of social reformation and return to the society, it enables on criminal to go back to the society after reformation. Secondly, it is preferred to exile, as well because exile does not prevent the danger by the criminal entirely, and the exiled person is about to commit other offences as a result of unemployment and lack of income. Thirdly, compared to whipping, which is one of the punishments in Iran, the imprisonment has better reformative effects since the whipping has passing effects, but the imprisonment has long stealing of more informative effects. Internationally imprisonment is more acceptable than whipping. Fourthly, the imprisonment is superior to penalty and remedy as the rich citizens can tolerate this punishment easily by paying the amount without little pressure. This payment does not bring about the least punishment for them, but the poor people do not afford this payment, so this punishment is not fair and is discriminative. Occasionally, this punishments cannot be carried out because of the defendant's inability to pay the penalty. In this case, the overall purpose, which is the same to remove and/ or abstain from the imprisonment, cannot be obtained. Therefore, if imprisonment is exerted with well observance of the regulations and principles, it is possible for the prisoner to reform and return to the society. This privileges cannot be overlooked since the prison is a place where the person can plan for the future by pondering about the past deeds. Therefore, through the evidence of the imprisonment, the proponents of this punishment believe that (4).

1-The judges may resort to punishment of the death, which has domestic and international consequences.

2-terrifying and debilitating the prisoner, which is considered during the exertion of the punishment, cannot come out true in practice.

3-It is not obvious how to treat with professional and dangerous criminals.

3- Opponents of Imprisonment

The opponents hold that short-term imprisonment creates a profound disturbance in the prisoner's life and endangers his employment and job, and his family is deprived from his presence. At large, many problems occur to the prisoners feeling of hostility is enhanced in the prisoner, the terrifying effect of the imprisonment fades away in him, so he does not feel regretful in reality. Secondly, the long-term imprisonment increases the danger of readjustment in the individual after release and the danger of disintegration of the prisoner's family. Consequently, the imprisonment is not able to control the society. Every punishment has its own philosophy of existence, and the main purpose of punishment is to account for the violation of rights, individual and social. The imprisonment, different aspects of which have attracted lawyers and psychologists, seems to violate the human rights itself. That is to say, putting somebody in a closed environment results in consequences which were not supposed to be produced. In other words punishment is supposed to reform the criminal and return him to the society as well as to punish him. However, imprisonment not only obtain these aims, but adds many negative consequences such as degrading the human dignity because in many countries, especially in Iran, the officers to manage prisons are usually prisoners not social workers, people without sufficient specialization and unfamiliar with the fundamentals of human rights, who humiliate the prisoner's dignity, with regard to the detention of the prisoner, if the prisoner is kept in a group cell, such issues as sexual

violence, formation of criminal team, epidemic of contagious diseases, addiction of the prisoner may occur. And if the prisoner is kept individually, he may afflicted with mental and emotional depression. Although individual cells with compulsory work and tough actions, etc. have became archaic, it cannot be denied that the systems of prison currently has shortcomings, and even a branch of radical criminology called annulism intends to annul the current criminal system, especially prisons. Therefore, the imprisonment has failed in a way and has not attained its purpose and philosophy of establishment; Therefore, the punishment of imprisonment must be avoided by replacing the measures that have better efficiency since prisons are not like schools, which is the more better. The large number of prisons does not indicate the cultural growth and society health, but it indicates that offence and crime have increased in that society. As a result of the above negative outcomes over the past decades, the imprisonment has changed in western countries and caused state men try to think up replacements for the imprisonment. Thus, they have embarked upon policing and planning the punishment of imprisonment by putting forward replacements for the imprisonment or substitute social measures with a modern approach to the imprisonment and usual punishment procedures and controlling the increasing population of the prisoners and preventing imposing excessive human and financial expenses, offering replacement punishment act over the past years and formation of special policing and planning headquarter are instances of this effort. The authorities of this policy maintain that the extreme perspective on crime by penal law makers, and the tendency of the law maker to anticipate this punishment in the law is one of the most important factors the crisis amelioration and increase in the penal population of the present prisons. Under such conditions, the authoritative organs for prisons and prison warding,which must basically be a place to keep, warn and reform the dangerous delinquent and criminals has turned to be a place to keep less important criminals, unintentional criminals, addicts and the financial deliquent who cannot afford the marriage portion and aliment. Therefore it is obvious that the prison, under such conditions, cannot act out its task, and respectful people with light offences must not be put in prison because this place is a place where people are trained badly, get unemployed, a place where the accountability is destroyed such that the professional prisoners are proud of this term. Of course, to decrease the usage of imprisonment does not mean the eradication of the punishment of imprisonment, but it is a comprehensive process the field of executive, judicial and criminal policy that attempts to prevent imprisoning the deliquent with unintentional trivial offences and financial debtors through anticipation of suitable replacement methods. Instead, it provides such people with a suitable opportunity to compensate for the damages arising from the offence, pay debts, reform the criminals of this kind in order to reduce the destructive efforts of the prison. Through this process, the judges and organs can choose whatever suitable for the individual and society by describing the type of the offence, personality and social status of the people and their penal precedence. Therefore, the exertion of this process prevents the people with petty offences from imprisonment and results in the decrease in the number of prisoners and increase in the rate of release.

Over the past two decades, in western and European countries, we have seen the exertion of these replacement for the imprisonment. As a result of studies on the social loss of the imprisonment, the western countries have attempted to implement replacement methods for the imprisonment and have encouraged the organs to do so.

4- Discussions

As mentioned, throughout the world the imprisonment is sentenced for most criminals in many countries. And many of those accused to penalty go to prison because they cannot afforded it. Prisons have abundant problems in many counties, especially in Iran, such as failure to allocate sufficient budget, failure to allot prison wardens. Sometimes, the term of the temporary detention is very long, and those who are put in prison are sentenced to imprisonment again after a while.

The released defendants are socially rejected and can never find a suitable job. Thus, owing to the abundant shortcomings such as crime creation and insufficiency in preventing hygienic problems, social, family and economic outcomes of the imprisonment, the penal policy markers do not think of replacement punishments and prison eradication. Most of the orders issued by the head of the judiciary encouraging the judges not to the punishment of imprisonment expect if necessary denotes this fact. Of course, the judges in Iran do not have a clear-cut framework for the sentences in order to use replacement Act submitted to the Islamic consultative Assembly in the mid 6000 has not been approved yet.

Although the exertion of the replacement punishments has not result in the population of the prisoners, and the enactment of these methods have forced problems, and these punishment are viewed petty and unreal by the public(6), the exertion of the replacement punishments such as doing common wealth activates require acculturation, and can be

reviewed as a suitable replacement in the future so that the penal policy makers can be assisted in the purposes and philosophy of punishments, especially imprisonment.

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Measuring Islamic-Driven Buyer Behavioral Implications: A Proposed Market-Minded Religiosity Scale

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Abstract: Islam is one of the major monotheistic religions in the world. Its importance is growing as the number of Muslim adherents is increasing, currently around 1.57 billion worldwide. The wealth of Muslim Arab countries is affecting the global economy as well as the international trade. Since religiosity has a major impact on consumer behavior, it was paramount to have an Islamic Behavioral Religiosity scale, measuring the degree of Muslims' religiosity from the behavioral perspective. The Islamic Behavioral Religiosity Scale (IBRS) could be used by marketers to assess the degree of consumers' religiosity and it could be linked to consumer behavior and work as a predictor to purchase patterns. The scale presented in this paper is a modification to the Islamic religiosity scale presented in 2007 by Chang-Ho C. Ji and Yodi Ibrahim. The scale was modified to include all divisions of Islam; belief, worship, legislation, and morality as well as behavior measurement. The modified scale was evaluated twice. First time was using a sample of 284 Muslim respondents in May and June 2010 and the second time was using 400 Muslim respondents in November and December 2010.
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Key words: Religion,Religiosity, Islamic religiosity scale, Islam.

1. Introduction:

The past religiosity scales were measuring religiosity per se (Faulkner & DeJong, 1966; Wilkes, Burnett, & Howell, 1986; Dudley & Kosinski, 1990; Hill & Jr. Hood, 1999; King & Crowther, 2004; Vitell, Paolillo, & Singh, 2005). Some of the Islamic religiosity scales were adapted from the Christianity scales (Ji & Ibrahim, 2007). Other group of literature measuring Islamic religiosity was not measuring the behavioral aspect or grouping respondents into clusters based on their religiosity degree (Krauss *et al.,* 2005; Tezcur & Azadarmaki, 2008; Tiliounine, Cummings, & Davern, 2009). The presented scale in the current research was adapted from Ji and Ibrahim (2007) scale, and then it was presented to experts in Islamic *Shari'ah,* sociologists, as well as marketing professors. Then quantitative survey was conducted to assess validity and reliability of the scale. The scale was validated and assessed twice. The first time was in May and June 2010 where the scale was presented to experts and then 256 surveys were used. The second time was during November and December 2010 where the scale was solved by 400 respondents.

Literature Review

The literature review section is divided into sub-sections. The first one is discussing the nature of Muslim consumers and their value. Secondly, religiosity is defined along with variations in religiosity degrees that lead to variations in consumer behavior. Thirdly, religiosity measurement attempts are presented. Fourth, the challenges facing researchers in measuring religiosity are presented.

Muslim Consumers

Islam is considered one of the major world religions and it originated 14 centuries ago. The Islamic market is a huge unsatisfied segment. It is estimated at about 1.4-1.8 billion consumers (Siddiqui, 2001;Sedgwick, 2006) [i] and 2.0 trillion dollars in 2010. The presence of oil in gulf countries has created wealth in that region and have attracted multi-national to locate in the gulf area *(*Al-Khatib*et al*., 2005; Anonymous, 2006). It has been forecasted and anticipated in about 20 years that third of world's population would be Muslims, two third of Muslim population would be 18 years of age (Quelch, 2001). The Muslim population is increasing with the rate of 1.84% annually.

Muslim consumers have a huge purchasing power in countries such as Egypt, Iran, India, Malaysia, Morocco, Turkey, Saudi Arabia and South Africa (Ba-Yunus & Siddiqui, 1998). The percentage of Muslims in the EU-15 is forecasted to be 10-15% by 2025, in 2006, it was 4.3%. In Germany and UK, Muslims are around 3% of the population; in France, it is around 9%. Muslims in Europe are diverse in terms of gender, age, and origins (Porter & Schwab, 2008). Muslims in Western Europe and US are growing minority however they are playing an active and effective role in the civil society and in affecting the governments of those countries. The growing percentage of Muslims in many countries worldwide,

have aroused people curiosity in better knowing and understanding the Islamic religion (Porter & Schwab, 2008). There has been an increase interest in Islam especially after the events of 11 September 2001. The information regarding Muslims and Middle East is currently available through media as there is an increase interest to write about this region and this religion(Khalili *et al*., 2002; Haque, 2004).

Marketing ethics bound by Islam is different than other marketing ethics as it has two major added aspects (Saeed *et al*., 2001). The first one is Islamic marketing ethics based on the principles mentioned in *Quran*, which is stable, fixed, and absolute and *Maqasid Ash Shari'ah*(Chapra, 2008). The second aspect is value maximization aim, Islamic marketing ethics aims at value maximization for the benefit of the whole society and not to merely maximizing profits (Saeed *et al*., 2001; Marinov, 2007). Even in financial services, there is an Islamic law, which governs and rules such services. An application of that is the interest, charging interest on credit is banned in Islamic law. Marketers cannot claim this feature in their financial service and promoting the interest is not accepted by the majority of Muslim consumers (Marinov, 2007).

Religiosity

One tool for measuring religion is through measuring religiosity. Religiosity is a "continuous rather than a discrete variable" (Beit-Hallahmi & Argyle, 1997). Religiosity is not an all or none question but every individual will have a certain degree of it. Religiosity is defined by McDaniel and Burnett (1990) as "a belief in God accompanied by a commitment to follow principles believed to be set by God." Another definition for religiosity is the condition or state of being religious (O'Brien & Palmer, 1993). Religiosity is not a unidimensional concept (Glock, 1962; Faulkner & DeJong, 1966; Allport & Ross, 1967; Stark & Glock, 1968; King & Hunt, 1972). It includes various elements of religion; namely belief, practice, knowledge, experience and the effects of those elements on daily activities (O'Connell, 1975).

Weaver and Agle (2002) had shown that religiosity has an impact on human behavior and attitudes. According to Hunt-Vitell model (1986, 1992), religiosity is one of the main personal elements embedded in the character of human beings. Religious self-identity has its impact on behavior, and this is shaped by the role expectations offered and defined by religion (Vitell *et al*., 2005). Religious affiliation and religiosity play a powerful and influential role in attitude formation (Hirschman, 1981), value choices (Keng & Yang, 1993) and is very much related to questions of why people consume (Hirschman, 1983).

Personal religiosity is a profound construct that aids in finding the differences in moral judgments, and this is referred back to the fact that religious ideology shapes people's judgments of what is right and wrong (Rest *et al*., 1986; Magill, 1992). Magill (1992) assumes that personal religiosity is what gives the explanations to the ethical nature of behavior. Religiosity is expected to have an effect on consumer's ethical beliefs. People who are more religious are more ethical in terms of their beliefs (Girogi & Marsh, 1990). Kennedy and Lawton (1998) had shown that there is a negative relationship between religiosity and behaving unethically. There have been numerous studies in an attempt to explain the effect of religiosity on behavior (Hunt & Vitell, 1986). One of the main propositions presented through their studies are; Personal deontological norms which are concerned with the basic moral ideals and teleological evaluations which are concerned with the situational influences (Hunt & Vitell, 1986, 1992).

In Hunt and Vitell (1986, 1992) research model where personal religiosity influences ethical judgment in at least three ways(As Cited in Clark & Dawson, 1996). Firstly, personal deontological norms composition is a function of religious belief and training, thus the individual's deontological evaluations may differ. Secondly, the relative importance of each individual philosophical viewpoint within the framework of his/her judgment process impacts both his/her deontological and teleological norms. People who are more religious give more weight to deontological considerations when evaluating situational ethical content (Rest *et al*., 1986; Hunt & Vitell, 1992; Clark & Dawson, 1996). Thirdly, religiosity imposes limitations on the considerations of alternative actions. Certain alternatives may be unacceptable to more religious people. Therefore, those alternatives are not expected to appear in the evoked set of actions. Hansen (1992) suggested that broad based ethical judgments are mainly used to screen and filter what would be accepted and what would not be accepted. Therefore, the impact of religiosity on selection is a significant matter. Teleological evaluations could be applied on the evoked set of alternatives, so one can deduce that the differences in perceived alternatives may result in differing ethical judgments.

Another assertive view of the effect of a person's religiosity on ethical considerations is the functionalist theory in sociology. This theory states that religion is a promoter to the norms enhancing social cohesion and religiosity is one of the effective determinants of people's values (Huffman, 1998).

Religiosity has the upper hand over beliefs and behaviors (Light *et al*., 1989). Theological dimension of ethics is a peculiar motivating power for human action and this dimension provides a motivation for moral action. Singh (2001) noted that by looking at various religious scriptures, books, teachings, one would find that all religions strongly motivate ethical behaviors and morality thus affecting people's behavior and decision-making.

Variations in Religiosity Degrees leading to Variations in Behaviors

Majority of the believers and worshippers have doubts. Thus, they differ in their commitments and religious degrees. This would explain why in the same religious group, there might be variances in religiosity measures (Beit-Hallahmi & Argyle, 1997). Muslims are divided into *Sunnis*, *Shi'tes*, and other subgroups; Christians are divided into Catholics, Protestants, and Orthodox Christians; Jews are divided into Hasidim, reform, and other subgroups. Another source of variations in the application of religion is the differences in political, legal, economic, social, linguistic, educational, attitudinal and religious beliefs and standards in every country (Melewar *et al.,* 2000). Saudi Arabia and Gulf countries, as an example, are theocratic countries claiming to be totally based on *Shari'ah*, whereas there are other Middle Eastern countries such as Egypt blending between Islam and civil law. On the political rights freedom scale, Bahrain, Egypt, Jordon, Lebanon and Yemen are rated as partly free whereas Iran, Iraq, Kuwait, Oman, Qatar, Saudi Arabia are rated as not free (Melewar*et al.,*2000).

There is a clear difference in religious degrees when studying Muslims. Some Muslims are adhering to all Islamic rules, i.e. seen as pious whereas others are more secular. The degree of commitment to Islam of both traditional and modern Muslims changes from extremely devout to merely nominal. Between those two poles, there are many levels describing the level of commitment of Muslims(Sedgwick, 2006). This depends on race, ethnicity, and social class of the individuals studied (Porter &Schwab, 2008). The common ground on which all Muslims agree upon is the monotheism baseline, i.e. the oneness of God and the prophet hood of Muhammad. *Quran* is differently understood and implemented, leading to differences in the implementation of Islam worldwide (Porter & Schwab, 2008). There are clear guidelines in Islam related to *Halal* and *Haram*(c.f. Al-Qardawi, 1999), and there are some debatable issues whereby Muslims are encouraged to think and debate on (Harold, 2004). There are several versions of *Shari'ah*, which produce different denominations of Islam (Sedgwick, 2006).

Religiosity Measurement Attempts

Studying religiosity is very complex and not an easy task as there is no single quality describing whether an individual is religious or as relatively or more religious than another individual (McGuire, 2002). There were various attempts and scales designed to measure religiosity. One of the prominent books in that field is *"Measures of Religiosity"*edited by (Hill & Jr. Hood, 1999) and (c.f. Faulkner & DeJong, 1966; King, 1967; King & Hunt, 1969, 1972;Genia, 1996; Peacock & Poloma, 1999; Khashan & Kreidie, 2001; Worthington *et al.,* 2003; King & Crowther, 2004;Ji & Ibrahim, 2007; Krauss *et al.,* 2007;Tezcur & Azadarmaki, 2008; Saat *et al.,* 2009; Tiliounine *et al.,* 2009; Sedikides & Gebauer, 2010). The following paragraphs are presenting some of the scales measuring religiosity per se.

Glock (1962) formed four dimensions to measure religiosity, which are ideological intellectual, ritualistic and experiential. In 1964, the consequential dimension was added(Glock & Nicosia, 1964). Those dimensions are religious beliefs, practices, knowledge, and experience. In addition, the fifth dimension would be consequential dimension, where Stark and Glock (1962) grouped the effect of the first four dimensions on the day-to-day lives of people. Faulkner and DeJong (1966) don't use the fifth dimension, as it does not highly correlated with the other dimensions. Many researchers do not use this fifth dimension in their studies (Faulkner & DeJong, 1966). Following is a brief explanation of the dimensions describing the religiosity term:

- The experiential dimension: is concerned with any feelings or sensations related to the communication with the divine essence. A feeling of the individual that he has been saved or cured from a certain disease, a feeling of a relationship with the sacred.
- The ritualistic dimension: includes religious practices such as worship, prayer, and participation in certain sacraments.
- The ideological dimension: refers to the content and scope of beliefs to which members of a religious group are expected to adhere.
- The intellectual dimension: includes the person's knowledge about the beliefs of his religion and the groups' religion as well as the sacred scriptures.
- The consequential dimension: includes how much religion, expressed in terms of religious beliefs, practices, and experiences, affects the individual's behavior in any context especially the non-religious contexts.

Allport and Ross (1967) constructed the intrinsic/extrinsic religiosity scale, which is using 5-points likert scale questions. The intrinsic scale is composed of eight items. The items are sentences like "I try hard to live my life according to my religious beliefs." The extrinsic scale contains six items and has sentences like "I go to religious services because it helps me to make friends" (Allport & Ross, 1967). Operationalizing the religiosity construct could be done through intrinsic and extrinsic variables. This is representing the backbone of empirical research in the psychology of religion (Kirkpatrick & Hood, 1990). Allport and Ross (1967) defined extrinsically motivated person as someone who uses his religion, and intrinsically motivated person comes in the other pole, which is the person who lives his religion. The nature of religious motivation of extrinsic person is personal and utilitarian. On the other hand, there is the intrinsic religiosity, which is motivated by internalized beliefs. The intrinsic people tend to develop a way of life matching those beliefs. It could be deduced that intrinsic religiosity have more impact on behavior than extrinsic religiosity, and intrinsic religiosity has a strong relationship with one's ethical beliefs. Extrinsic religiosity does not involve spirituality, but is concerned with how social networks perceive one's religion, and how this religion would comfort and make the individual at ease (Vitell *et al.,*2005). In Islam, only concentrating on extrinsic religiosity is considered as *Riya'*, which is a sinful act and unislamic. However, for the sake of linking between religiosity and behavior both intrinsic and extrinsic religiosity measures were included in the scale modified for the purpose of the current research.

Every question used in Allport's Intrinsic-Extrinsic scale has the phrase such as "the only reason" or "the main reason" reflecting the elements related to the core of the personal's religiosity. Without those indicators, one cannot differentiate between the phrases related to intrinsic and the ones categorized as extrinsic (Gorsuch *et al.,* 1997). With those statements, respondents would choose what is best describing their core and central reason for being religious (Spilka *et al.,* 2003). One of the main flaws in the intrinsic/extrinsic model is that extrinsic items are not exactly on the other side of intrinsic items. They did not correlate negatively with the intrinsic items (Spilka et al, 2003). For example, using the phrase "the only reason" was not enough for respondents to treat the intrinsic and extrinsic as mutually exclusive as people are normally not bounded very much by logic and reasoning especially in religious matters. Kirkpatrick and Hood (1990) had explained the reasons of the insufficiency of extrinsic scale and the reasons why it has low internal consistency and reliability. They mentioned that the scale was composed of two different ways in measuring extrinsic people. The first was related to receiving a personal reward or benefit such as comfort during stress. Secondly, is social and it focuses on the people that the individual dealing with when practicing religion. This would also indicate and reflect building up relations and making friends and thus would be termed extrinsic social. During 1980s and 1990s it was found that the versions of intrinsic/extrinsic designed by Allport is not sufficient and it needs modifications (Spilka *et al.,*2003). The extrinsic dimension was seen as being very complicated and rated low in reliability. In addition, extrinsic dimension thoroughness and attention to details were also investigated.

Allport and Ross (1967) have modified the statements and items used for intrinsic versus extrinsic. Instead of dealing with them as ends of single dimension, they have dealt with them as two concrete and different dimensions each with its own separate set of definitions, elements, and items. Allport *et al.* (1982) tried to separate between intrinsic and extrinsic and make a clear differentiation between them. However, scholars noted that this differentiation and distinction is not sufficient (Spilka *et al.,* 2003). People who are categorized as intrinsic might be prejudiced and stereotyped if they felt that religion was something that is personal and exclusionary, however if they have regarded religion as an open quest then they would be less judgmental. Batson, Ventis and Larry (1993) have added the quest dimension, which would differentiate and distinguish between intrinsic and extrinsic. People, who are mainly very intrinsic and very committed to their faith, do not regard religion as an ongoing quest and till now this hypothesis is valid and true (Spilka *et al.,*2003). However, still the majority of religiosity scales does not include the quest section and concentrate more on intrinsic and extrinsic questions.

Intrinsic and extrinsic are different in their ethical evaluations based on the following reasons (Hunt & Vitell, 1986). First, they differ in perceptions of reality. Every individual perceives reality in a different way leading to a variation in ethical evaluations. Perception of reality could be analyzed into perception of available alternatives, perceived results of those alternatives and differing evaluations and assessments of the probability of occurrence. Secondly, different teleological evaluations, the perceived attractiveness of outcomes and results may differ among individuals. Some individuals may place high values on the good of the company, others on different stakeholder groups, or consumers in general. Hamby (1973) has found that intrinsic people are more concerned with

interpersonal relationships. They care for social customs and they like to control their impulses regarding others. Therefore, religious person might have stronger utilitarian norms and values than non-religious and might judge actions and behaviors from a less egoistic perspective. Thirdly, differing deontological evaluations, which from Hunt and Vitell (1986) perspective involve comparing the questioned behavior with a set of pre-determined personal values or rules. Rokeach (1969) illustrated that religious people have a value system that is totally different from the less or non-religious people. In some of the specific moral values, Rokeach mentioned that religious people rate higher in the values related to forgiveness and obedience when compared to less religious people.

Another source of variation, not mentioned by Hunt and Vitell (1986), is decision making style of the respondents (Mayo & Marks, 1990). Pargament *et al.* (1988) mentioned that problem solving styles depends on intrinsic religiosity. There is an increasing dependence on the deity to help in the decision making process. Problem solving techniques vary from the religious to non-religious person (Vitell *et al.,* 2005). Religious person uses God help in the decision making and is more passive in issues and situations that need ethical judgments, whereas the less-religious would be more active, self-dependent and self-reliant. Extrinsically religious consumers put more weight on brand names and well-established stores, as they are trendier than intrinsically religious consumers. The intrinsically religious consumers are conservative and traditional and seeking sales promotions and offers and they are less innovative and trendy (Essoo & Dibb, 2004).

Another way for measuring religiosity was done through measuring a 3-item measure developed by Wilkes *et al.*(1986) and was then further developed:

- I go to church regularly
- Spiritual values are more important than material things
- If Americans were more religious, this would be a better country.

There are numerous scales attempting to measure Islamic religiosity (c.f. Worthington *et al.,* 2003; Essoo & Dibb, 2004; Krauss *et al.,* 2005;Ji & Ibrahim, 2007; Rehman & Shabbir, 2010)[ii]. Essoo and Dibb (2004) used intrinsic and extrinsic religiosity measures. Ji and Ibrahim (2007) also used intrinsic and extrinsic religiosity measures, added Islamic doctrinal questions, and a quest section. Krauss *et al.* (2005) developed Muslim Religiosity Personality Measurement Inventory (MRPI), which assumes that Islamic religiosity can be understood through two main constructs. Those two constructs are Islamic worldview and religious personality. Islamic worldview reflects the belief in Islamic creed (*Aqidah*), which is laid by the *Quran* and *Sunna*. Religious personality is manifested through one's religious worldview in worship *(Ibadah)* or how person expresses his/her traits and behaviors with respect to religion. Rehman and Shabbir (2010) used Glock and Stark's (1964) religiosity dimensions after modifying it with Islamic rules. Worthington *et al.* (2003) designed a scale named religious commitment inventory measuring the degree to which a person adheres to his/her religious values, beliefs, and practices and uses them in daily living.

All the mentioned scales were not implemented in Egypt. For that reason, it was crucial to test the applicability of one of those scales on Egypt. Further, most of the scales developed are composed of at least 45 variables, which constitute an obstacle for the researcher. Thus, another reason for modifying an already existing scale is to run factor analysis to reduce the variables to minimum level to be able to include it in later phases of empirical study (Hair *et al.,* 2006). Another reason for the modification is Islamic religiosity needs to be assessed from behavioral perspective to be linked with consumer attitudes. The following sections explaining the obstacles facing researchers in measuring religiosity and the methodology followed to reach an Islamic Behavioral Religiosity Scale.

Challenges in Measuring Religiosity

It is difficult to gather information from consumers regarding their religious beliefs and behaviors, as this kind of information is regarded as personal and confidential. The majority of religious behaviors are private and not shared in public. Researchers depend on self-report methods which sometimes might be misleading or not precise (Beit-Hallahmi & Argyle, 1997). Some respondents might manipulate the answers for social desirability reasons.

Researchers might overcome this problem by using the following two methods (Beit-Hallahmi & Argyle, 1997). First, using variety of sources to gather religious information, then compare between the results from those sources to make sure that all answers are consistent. Secondly, noting changes across time and across space as well in response to the same questions. The main aim of doing that is to make sure that the responses are far from any kind of mechanical or personal involvement or biases. Noting an action or reaction that would be considered as socially accepted and desired in a certain point in time is by itself an important social indicator. Another problem facing researchers in relation to measuring religion is construct validity (Beit-

Hallahmi & Argyle, 1997). Construct validity is reflected through changes in the answers of respondents, and by changes and variations in the religiosity, which would follow theoretical predictions. This could be solved through using other religiosity measures as well as other non-religious behaviors.

It is very difficult methodologically to figure out and describe people's religion. The sociological statistics data such as census, opinion surveys and generated figures from institutions as membership rolls might be misleading. Questionnaires can be used to describe religion of consumers by directly asking the respondents about their religious affiliation or preference. For example, asking the respondents whether they are protestant, Catholics, Jew, Muslim(Beit-Hallahmi & Argyle, 1997). Questionnaires are not the only means for psychologists to collect information regarding religion. They can depend on other means such as interviews, which should be carefully designed and administered, projective tests, defining and observing religious behavior. The prevailing methodology used by psychologists in measuring religion is the associational technique. Variables are associated to be able to define religious people, for example, parents who take their children to church, this would make their children more religious, but it will not hold true that attending church produces religious children. It could be the modeling of religion by the parents in the home that is important, or the social networks created through church attendance (Spilka *et al.,* 2003).

The above section is the literature review, the following paragraphs are concerned with the research methodology adopted to adapt and modify and construct an Islamic Behavioral Religiosity Scale (IBRS).

2. Research Methodology

Research methodology is based on the mixed method using both quantitative research through expert interviews and qualitative research through surveys (Deshpande, 1983).The presented scale in the current research was adapted from Ji and Ibrahim (2007) scale, and then it was presented to experts in Islamic *Shari'ah,* sociologists, as well as marketing professors. The quantitative survey was conducted to assess validity and reliability of the survey. The scale was validated and assessed twice. The first time was in May and June 2010 where the scale was presented to experts and 256 surveys were collected. The second time was during November and December 2010 where the scale was solved by 400 respondents.

Expert Interviews

In designing Islamic Behavioral Religiosity Scale, the researcher selected to adapt and modify the scale developed by Chang-Ho C. Ji and Yodi Ibrahim (2007). This is a recent scale at the time of conducting the study. It was based on Allport and Ross (1967) religiosity scale, which is the widely used. It is divided into four main variables: Islamic doctrinal, intrinsic religiosity, extrinsic religiosity, and quest. This division enables measuring religiosity from behavioral perspective as weights could be added to every variable. Extrinsic religiosity is related to socially accepted religious behaviors. Extrinsic religiosity from Islamic perspective is considered *Riya'* and depending on it fully is considered a major sin. It was included in the scale as it was shown through previous studies that consumers' degree in intrinsic and extrinsic religiosity has major implications on behavior. This scale was selected also based on the input from experts in religion as they agreed that religion, especially in Egypt could be divided into core religion, which is translated into intrinsic religiosity and people using religion, which is translated into extrinsic religiosity.

This scale was shown to experts through in-depth interviews. The interviews were conducted with a sociologist, two *Shari'ah* scholars, and a marketing professor. Pandeli (2010) prominent sociologist working at American university in Cairo commented when designing a religious scale, one has to differentiate between behaviors, beliefs and intentions, as there is a major difference between what a person believe in and his/her behavior. Ibrahim (2010) a prominent *Shari'ah* scholar mentioned that constructing an Islamic Behavioral Religiosity Scale is impossible as religiosity and spirituality are intangible matters. Religiosity and spirituality might be measured by adding to them some tangible features. Islam is divided into belief, worship, legislation as well as morality (El-Bassiouny *et al.,*2008). Another *Shari'ah* scholar, who preferred to remain anonymous, commented that the adapted scale is missing more questions related to doctrinal orthodoxy, Islamic dealings, and morality as the adapted scale was concentrating more on beliefs and worship. The sociologist along with the *Shari'ah* scholar agreed that the scale should include sections regarding social, cultural, and educational and marriage aspects from Islamic perspective. Further, experts recommended removing the quest section from the adapted scale, as it does not fit with Islamic religion. Based on experts' comments, the Islamic religiosity scale was modified. Scale was targeting Muslim Sunni and not the other Islamic groups as the research is implemented in Egypt and Sunnis are dominant in Egypt. Further, *Sunnis* are 85%

worldwide whereas *Shi'a* is 15% (Esposito & Mogahed, 2007).

After modifying the scale based on the above-mentioned comments, it was shown to Professor Pallub, professor of Marketing in Daniels College of Business, to reassure the design of the scale as well as content validity. Based on his comments, another question was added to the scale, which is measuring the weight of every variable under Islamic Behavioral Religiosity Scale. By this question, the behavioral aspect could be measured. The main objective of such a question is to find the weight (a, b, c) multiplied by every construct to find the overall degree of religiosity.

Islamic Behavioral Religiosity Degree = a*Islamic doctrinal + b*intrinsic religiosity + c*extrinsic religiosity.

Quantitative Research

After modifying the Islamic Behavioral Religiosity Scale based on experts' comments, surveys are administered to run factor analysis. Piloting was administered first to collect consumers' feedback, to check language of the scale as well as the organization of the scale. There were not major comments and the scale was not modified based on consumers. Following piloting phase, survey was administered through the Internet[1]. The sampling technique used to validate Islamic Behavioral Religiosity Scale was non-probability snowballing technique. The scale was sent to respondents via email and they were asked to forward it to their network. Probability sampling was difficult to administer due to sensitivity of the topic, it is difficult to know respondents' religion without asking them, and this is not accepted in Egyptian culture. The first run of the research was administered in May and June 2010 whereas the second run was administered in November and December 2011.

Factor analysis measurement is performed to reduce the variables per religiosity factors, as Islamic Religiosity Scale was composed of 42 variables (Hair *et al.,* 2006). Further, reliability and validity tests were performed. The sample size needed for testing the scale was 210 as at least 5 respondents per variable are needed (Hair *et al.,* 2006; Malhotra, 2010). In the first run of the survey, the researcher got 348 questionnaires whereas 256 were valid. The response rate is 73.4%. The 348 respondents answered the questions related to Islamic doctrinal, 288 completed intrinsic religiosity section, 256 completed the entire questionnaire. Basic demographic information related to sample unit is being a Muslim. Other demographic information such as age, gender, and occupation were not basic selection criteria. The reason behind this selection is related to the main objective of running this factor analysis, which is testing Islamic Behavioral Religiosity Scale. As for the second run of the survey, 631 respondents filled out the survey, however only 417 were valid. Thus, the response rate for this re-run is 66%.

The scale used is non comparative, itemized rating scale and questions are using likert scale (Malhotra, 2010). A measurement scale of 5-point likert scale ranging from "strongly disagree" to "strongly agree" which requires the respondents to indicate a degree of agreement or disagreement with each of a series of statements related to the stimulus objects.

Religiosity Scale Validation

Kaiser Meyer Olkin (KMO) measure of sampling adequacy had been calculated to determine the feasibility of running a factor analysis on the adapted scale. KMO approaching 1 generally indicates that factor analysis is useful with the data; approaching zero indicates the result of the factor analysis will not be very useful(Hair *et al.,* 2006). Bartlett's test significance indicates whether the correlation matrix is an identity matrix, which what indicates that the variables are unrelated. The first run for the scale KMO was 0.890 and the significance was 0.000. The researcher determined three factors while doing the confirmatory factor analysis; this was based on previous studies. Factor analysis and factor loading used is more than 0.6 (C. Ji & Ibrahim, 2007). First Factor Islamic doctrinal explains 22.035%; second factor intrinsic religiosity explains 13.806% and third factor extrinsic religiosity explains 9.378%. The cumulative rotation sum of squared loadings for the three factors would be 45.578%, which is an acceptable figure. More than 40% of the variables in the scale reflected in those three factors. As for the second run for the scale KMO is 0.886 and significance is 0.000.

The rotated component matrix is used for analysis, as it is better in data reduction. Based on past studies and the knowledge of the researcher, the factors could be labeled as: factor 1: intrinsic religiosity, factor 2: Islamic doctrinal, factor 3: the extrinsic religiosity. The first eight variables loaded with factor 2. The ninth, twenty-third, twenty-seventh, twenty-eight variables did not load with any factor, therefore they are deleted from the scale. The 10th to the 31st variables load with factor 1 (except for

[1] www.surveymonkey.com

23rd, 27th, 28th). Variables 31st, 36th are added to intrinsic religiosity[2]. Variables from 32nd to 42nd except for 31st and 36th are added to extrinsic religiosity. As for 33rd variable, it could be removed as it is loading on both intrinsic and extrinsic religiosity. The composite sum was computed to calculate overall Islamic doctrinal, overall intrinsic religiosity, overall extrinsic religiosity as well as the overall religiosity for the sample. The composite sum was calculated based on the new distribution of variables over factors. Strongly disagree was translated into one whereas strongly agree is translated into five. Mean for composites of Islamic Doctrinal was 4.89 inclined toward strongly agree; composite of intrinsic religiosity is 3.7664 inclined toward agree, composite of extrinsic religiosity 2.7135 inclined toward neutral, composite of overall religiosity 3.7 inclined toward agree.

Validity and Reliability

The content validity was achieved by asking experts in the field. Content validity "consists of a subjective but systematic evaluation of the representativeness of the content of a scale for the measuring task at hand" (Malhotra, 2010). Face validity was achieved through piloting the survey with experts and some consumers. Construct validity which is a "a type of validity that addresses the question of what construct or characteristic the scale is measuring, (where) an attempt is made to answer theoretical questions of why a scale works and what deductions can be made concerning the theory underlying the scale" (Malhotra, 2010). Construct validity is divided into convergent, discriminant, and nomological validity. According to Bagozzi and Foxall (1996), both convergent and discriminant validity can be assessed by using factor analysis. Confirmatory factor analysis was implemented during another re-run for the validation and reliability of the new scale.

The last type of validity is construct, which "addresses the question of what construct or characteristic the scale is, in fact, measuring" (Malhotra, 2010). Construct validity includes convergent, discriminant and nomological validity. Convergent validity is "the extent to which the scale correlates positively with other measures of the same construct" (Malhotra, 2010). Discriminant validity "is the extent to which a measure does not correlate with other constructs from which is supposed to differ" (Malhotra, 2010, p. 321). Nomological validity is "the extent to which the scale correlates in theoretically predicted ways with measures of different but related constructs" (Malhotra, 2010). According to Bagozzi and Foxall (1966) and Hair *et al.*(2006), both convergent and discriminant validity are assessed by using factor analysis as well as confirmatory factor analysis. Confirmatory factor analysis is calculated for the Islamic Behavioral Religiosity variable, which is presented in the following section.The above-mentioned methodology is following the model presented by Churchill (1979).

Confirmatory Factor Analysis

To measure construct validity of Islamic Behavioral Religiosity Scale, confirmatory factor analysis was implemented using AMOS software. CFA reflects how well the measured variables represent the constructs (Hair *et al.,*2006). Islamic Behavioral Religiosity scale was composed of Islamic doctrinal, intrinsic religiosity, extrinsic religiosity. Following is figure of confirmatory factor analyses before modification.

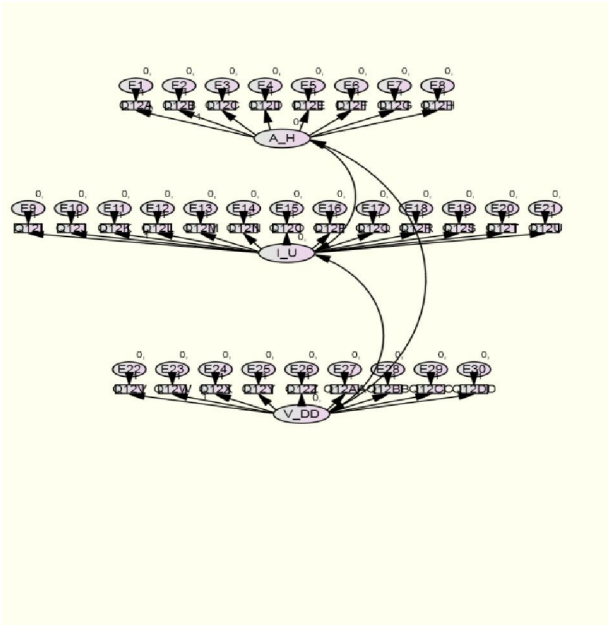

Figure 1: Path Diagram before Modification

As for regression weights per variable, analyzing the P value, it was found that the variables are highly significant as it is less than 0.001. This reflects that every statement per variable reflects the variable itself. Further, estimates are positive sign, which also reflects that every statement is representative of the variable. As for standardized regressionweights should be more than 0.5, thus the variables less than 0.5 were excluded from the survey. Islamic doctrinal and intrinsic religiosity

[2] Please refer to Appendix 1 for adapted religiosity scale and Appendix 2 for the new proposed scale.

variables are the same whereas some items are removed from extrinsic religiosity variable. Based on the CFA, some questions from extrinsic religiosity were removed whereas for the other factors, which are Islamic doctrinal and intrinsic religiosity, nothing was removed from the items. After removing the items with less than 0.5 in standardized regression weight, another confirmatory factor analysis is implemented. Following are the results of the modified confirmatory factor analysis.

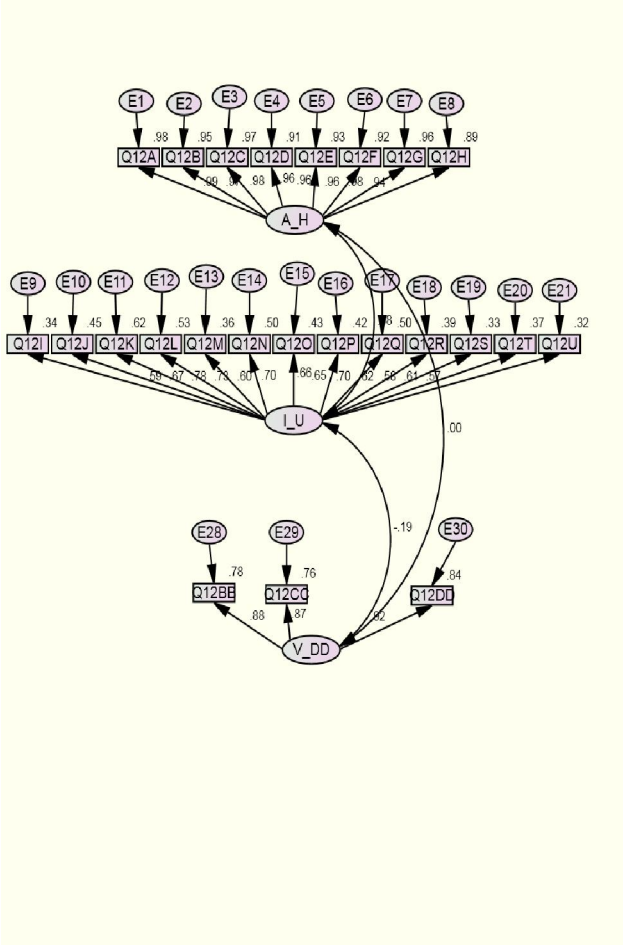

Figure 2: Path Diagram after Modification

Another measure was calculated to ensure convergent validity that is variance extracted and it is calculated for the model after modification. "Variance extracted among a set of construct items is a summary indicator of convergence" (Hair *et al.,* 2006). Variance extracted for questions related to Islamic doctrinal is 0.9375, for intrinsic religiosity is approximately 0.45, for extrinsic religiosity is 0.8. The accepted variance extracted should be 0.5 or more as this reflects adequate convergence (Hair *et al.,* 2006). Thus, the variance extracted per variable is accepted, whereas the intrinsic religiosity is 0.45, which is near the acceptance threshold.

As for model after modification fit measures are as follows:

Table 1: Model After Modification Fit Measures

Model	NFI Delta1	RFI rho1	IFI Delta2	TLI rho2	CFI	RMSEA
Default model	.801	.760	.818	.779	.817	0.14
Saturated model	1.000		1.000		1.000	
Independence model	.000	.000	.000	.000	.000	0.298

Normed fit index (NFI) is "one of the original incremental fit indices. It rangesbetween 0 and 1 and a model with perfect fit would produce an NFI of 1" (Hair *et al.,* 2006). NFI for the model is 0.801. Comparative fit index (CFI) "is an incremental fit index that is an improved version of the normed fit index (NFI). CFI is Normed so that values range between 0 and 1, with higher values indicating better fit" (Hair *et al.,* 2006). CFI for the model is 0.817. Tucker Lewis Index (TLI) "predates the CFI and is conceptually similar in that it also involves a mathematical comparison of a specified theoretical measurement model and a baseline null model" (Hair *et al.,* 2006). Models with values approaching 1 are good fit (Hair *et al.,* 2006). Root mean square error of approximation (RMSEA) is a measure attempting in correcting the tendency of X^2goodness of fit test statistics in rejecting models with large samples or a large number of observed variables (Hair *et al.,* 2006). Lower RMSEA values indicate better fit. In the current model, it is 0.14.

An added question to the modified scale was inserted in the survey to measure the weights of every factor reflecting Islamic Behavioral Religiosity Degree. This question enabled the researcher in constructing the following equation by which religiosity degree could be calculated from the behavioral perspective. Following is the equation used to measure Islamic Behavioral Religiosity Degree:

Islamic Behavioral Religiosity Degree=55.86*Islamic Doctrinal + 26.25*Intrinsic Religiosity + 17.89 * Extrinsic Religiosity

Reliability refers to "the extent to which a scale produces consistent results if repeated measurements are made"(Malhotra, 2010). "The relationship between reliability and validity can be understood in terms of the true score model. If a measure is perfectly valid, it is also perfectly reliable" (Malhotra, 2010). Assessing reliability is done through test-retest, alternative forms, and internal consistency models. Internal consistency is "used to assess the reliability of a summated scale where several items are summed to form a total score" (Malhotra, 2010). Internal consistency could be either assessed by split-half reliability or through the coefficient alpha or Cronbach's alpha (Malhotra, 2010). "Cronbach alpha is the average of all possible split half coefficients resulting from different ways of splitting the scale items. The coefficient varies from 0 to 1, a value of 0.6 or less generally indicates unsatisfactory internal consistency reliability" (Malhotra, 2010)

First run reliability coefficient Cronbach alpha, for Islamic doctrinal was calculated to be 0.94, for intrinsic religiosity was 0.916 and as for extrinsic religiosity was 0.819. Re-run overall reliability for overall religiosity was 0.847. Cronbach alpha for Islamic doctrinal is 0.991, for intrinsic religiosity is 0.902 and for extrinsic religiosity is 0.842.

3. Results and Discussion

Significant Differences between Nominal Variables and Religiosity

Cross tabulations have been run between demographic and composites using T-test and ANOVA analyses. The univariate techniques for analyzing group differences are the t-test within two groups and analysis of variance (ANOVA) for two or more groups (Hair *et al.,* 2006). T-test "is a test to assess the statistical significance between two groups on a single dependent variable" (Hair *et al.,* 2006). ANOVA is "statistical technique used to determine whether samples from two or more groups come from populations with equal means." (Hair *et al.,* 2006). In the first run of the scale, it was shown that there is no significant difference between demographic variables and religiosity variables as well overall religiosity construct.

In the second run of the scale, it was shown that there is significant difference between gender and intrinsic religiosity. The mean of the answers for those questions was inclined more to male more than female. There was no significant difference between gender and extrinsic religiosity, Islamic doctrinal and overall religiosity. There is a significant difference between gender and intrinsic religiosity, intrinsic religiosity weight, and extrinsic religiosity weight. The mean for the responses of male is more than that for the female in all those variables except for extrinsic religiosity weight and extrinsic religiosity. This information is crucial for marketers in as they need to segment their targets based on religiosity and gender as well as there is significant difference between males and females for some religiosity variables.

There is significant difference between age groups and intrinsic religiosity where the highest mean was age group above 50 and the least was age group 30-40. There is significant difference for extrinsic religiosity where the highest mean was age group 21-30 and the least was age group 40-50. There is significant difference for Islamic behavioral religiosity degree equation where the least mean is for age group 30-40 and the highest mean is for age group 40-50. There is significant difference between income and intrinsic religiosity where the highest mean is for average income and the lowest is for above average group. There is significant difference between income and extrinsic religiosity where the highest mean is for wealthy and the lowest mean is for average income.

Cluster Analysis Based on Islamic Behavioral Religiosity Scale

Cluster analysis is a type of multivariate techniques whose primary purpose are to group objects based on the characteristics they posses. Cluster analysis "classifies objects so that each object is similar to others in the cluster based on a set of selected characteristics. The resulting cluster of objects should exhibit high internal (within-cluster) homogeneity and high external (between-cluster) heterogeneity" (Hair *et al.,* 2006).

Using the Islamic Behavioral Religiosity formula, two clusters were found amongst the respondents in the re-run phase. Following are the clusters after iteration:

Table 2: Religiosity Cluster after Iteration

	Cluster	
	1	2
Overall Islamic Behavioral Religiosity Degree	3.70	4.48

This reflects that there are two clusters. The first cluster is agreeing with the statements of Islamic Behavioral Religiosity Scale (3.7), the second one is strongly agreeing (4.48) with the same statements. The respondents could be divided into moderate and high. Moderate are the ones who agree with all the statements of religiosity scale and high are the ones who strongly agree with all the statements. Moderates believe in the five pillars *Arkan* of Islam, they agree on behaviors directly related to the core of religion and they agree on religious behaviors done to leave

positive impression among society members. As for the high, they strongly agree with Islamic doctrinal, intrinsic religiosity as well extrinsic religiosity. This is an added contribution to the literature, as the pervious scales did not group samples based on religiosity degree or implement cluster analyses. Therefore, religiosity clusters work as an important segmentation criteria for marketers as there is significant difference between behavior of moderate and high religiosity clusters. Every cluster is expected to behave in a different ways per variable.

Implications for Future research

The main objective of performing factor analysis to the religiosity scale is data reduction. The new Islamic Behavioral Religiosity scale would be used as part of future studies performed by the researcher. This new scale would be correlated with the consumer behavior and attitude towards Islamic products/services e.g. Islamic banks. The aim of that correlation is to find whether consumer behavior changes if religiosity degree changes or not.

The scale could be tested on different samples of Muslims in different regions of the world. In the meantime, the proposed scale could be linked with the behavior. It could be part of future studies implemented by marketers and academics, where the research would assess the link between consumer behavior and attitude towards products/ services. Future studies could test whether the degree of religiosity has an impact on the behavior and there is significant difference between the highly intrinsic person and the highly extrinsic person in terms of the behavior.

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Appendix 1

Please indicate your level of agreement with the following statements:

Rotated Component Matrix[a]

		Component		
		1	2	3
1.	I believe that prayer (*Salat*) as ordained by God and his prophet is crucial to the life of a Muslim		.750	
2.	I believe and declare that no other God but Allah and Mohammed is God's final prophet		.895	
3.	I believe that fasting during the holy period of Ramadan is one of the responsibilities of Muslims that is important to do		.813	
4.	I believe that hajj pilgrimage for those with the privilege to do so is important in the life of a Muslim		.765	
5.	I believe that there will be the end of time when people will be judged according to how they live their life in this world		.787	
6.	I believe in the reasons of having human beings life and the ability to select a way of life either based on God's principles leading to eternal heaven or the principles God warned us against leading to eternal hell		.665	
7.	I believe that Koran is the word of Allah, thus its authority is justified		.873	
8.	I believe that almsgiving (Zakat) is very important in the life of a good Muslim to be paid every year		.810	
9.	I am aware of the *Masarefelzakat*(or the Zakat channels; where I should pay my Zakat)			
10.	Religion is especially important to me because it answers many questions about the meaning of life.	.697		
11.	I read the literature and books about my Islamic faith.	.646		
12.	I watch Islamic programs on TV or listen to religious programs on radio.	.744		
13.	I believe that more Islamic programs, channels should be offered	.687		
14.	I try to carry my religion over into all my other dealings in life.	.671		
15.	I believe that teaching Islam in schools should be obligatory.	.534		
16.	I will send my children to Islamic schools and not to secular schools.	.681		
17.	I will bring a sheikh to teach my children the Islamic principles.	.545		
18.	I am aware of God's obligations and guidelines when I deal with my family members (parents, brothers, sisters, uncles, aunts, cousins, siblings.. Etc)	.445		
19.	I believe that veil is obligatory	.625		
20.	I am veiled (for female respondents)/ I would enforce the veil on my wife (for male respondents)	.555		
21.	I read the Islamic literature and the Islamic books about the equal rights to man and woman in "affection and mercy"	.580		
22.	My religious beliefs are what really lie behind my whole approach to life	.621		
23.	If not prevented by unavoidable circumstances, I donate money as sadaka to the needy people			
24.	If not prevented by unavoidable circumstances, I pray at the mosque	.609		
25.	I believe that praying at the mosque is an added benefit rather praying at home or at work	.620		
26.	It is important for me to spend periods of time in private religious thought and meditation	.598		
27.	I seek and work to obtain Allah's grant of material return (rizk) without cheating, hoarding and squandering			
28.	I abstain myself from giving or taking Riba as defined in the exchange of material monetary units			
29.	I believe that praying Sunna, Nawafil, Qiyam Elleil are extremely beneficial for Muslims	.693		
30.	I always pray Sunna, Nawafil, Qiyam Elleil	.591		
31.	One reason for my being a member of a Mosque (or attending religious sessions) is that such membership helps to establish a person in the community	.600		
32.	Although I am a religious person, I refuse to let religious considerations influence my everyday affair			.659
33.	I think that the political constitution should be secular and not based on the *Shari'a*h	-.528		.415
34.	A primary reason for my interest in religion is that my mosque or my affiliation with a religious group has pleasant social activities			.659
35.	Occasionally I find it necessary to compromise my religious beliefs in order to protect my social and economic interest			.601
36.	The mosque or attending religious meetings is most important as a place to formulate good social relationships	.491		
37.	Although I believe in my religion, I feel there are many more important things in life			.576
38.	It does not matter so much what I believe as long as I lead a moral life			.634
39.	I pray mainly because I have been taught to pray			.624
40.	The primary purpose of prayer is to gain relief and protection			.650
41.	What religion offers me most is comfort when sorrows and misfortune strike			.610
42.	The purpose of prayer is to secure a happy and peaceful life			.644

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

a. Rotation converged in 5 iterations.

Appendix 2
Modified Religiosity Scale

Divide 100% on the weight of importance of the following Islamic Items:

1.	I believe that prayer (salat) as ordained by God and his prophet is crucial to the life of a Muslim
2.	I believe and declare that no other God but Allah and Mohammed is God's final prophet
3.	I believe that fasting during the holy period of Ramadan is one of the responsibilities of Muslims that is important to do
4.	I believe that hajj pilgrimage for those with the privilege to do so is important in the life of a Muslim
5.	I believe that there will be the end of time when people will be judged according to how they live their life in this world
6.	I believe in the reasons of having human beings life and the ability to select a way of life either based on God's principles leading to eternal heaven or the principles God warned us against leading to eternal hell
7.	I believe that Koran is the word of Allah, thus its authority is justified
8.	I believe that almsgiving (Zakat) is very important in the life of a good Muslim to be paid every year
9.	Religion is especially important to me because it answers many questions about the meaning of life.
10.	I read the literature and books about my Islamic faith.
11.	I watch Islamic programs on TV or listen to religious programs on radio.
12.	I believe that more Islamic programs, channels should be offered
13.	I try to carry my religion over into all my other dealings in life (human dealings with my network (family members, friends and colleagues…Etc) and financial dealings).
14.	I will send my kids to Islamic schools and not to secular schools.
15.	I believe that veil is obligatory
16.	I am veiled (for female respondents)/ I would convince my wife to get veiled (for male respondents)
17.	My religious beliefs are what really lie behind my whole approach to life
18.	If not prevented by unavoidable circumstances, I pray at the mosque
19.	I believe that praying at the mosque is an added benefit rather praying at home or at work
20.	I believe that praying Sunna, nawafil, qiyamelleil are extremely beneficial for Muslims
21.	I always pray Sunna, nawafil, qiyamelleil
22.	One reason for my being a member of a Mosque (or attending religious sessions) is that such membership helps to establish a person in the community
23.	Although I am a religious person, I refuse to let religious considerations influence my everyday affair
24.	A primary reason for my interest in religion is that my mosque or my affiliation with a religious group has pleasant social activities
25.	Occasionally I find it necessary to compromise my religious beliefs in order to protect my social and economic interest
26.	It does not matter so much what I believe as long as I lead a moral life
27.	I pray mainly because I have been taught to pray
28.	The primary purpose of prayer is to gain relief and protection
29.	What religion offers me most is comfort when sorrows and misfortune strike
30.	The purpose of prayer is to secure a happy and peaceful life

Divide 100% on the weight of importance of the following Islamic Items:

Knowledge regarding the *Aqidah* and the 5 pillars of Islam	
Behaviors directly related to the core of religion (like *FiqhElmoamalat* and praying at the mosque)	
Religious behaviors done for the social desirability purposes	
Total	100

[i] There is no accurate statistics reflecting the current number of Muslims. This is based on T.C. Melewar speech in ICIMB. In population reference bureau and CIA factbook it was mentioned that Muslim's population is 1.6 (http://www.prb.org/Articles/2011/muslim-population-growth.aspx; https://www.cia.gov/library/publications/the-world-factbook/)

[ii] This information was assimilated from the http://icimb.um.edu.my/ (first international Islamic marketing conference held in Malaysia in 2010). The main theme of the conference was Islamic marketing and Islamic Religiosity, however the scales mentioned in the conference are not yet published.

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A study of the effects of an aerobic training program on the social maturity of male non-athlete students: the case of an Iranian experience

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Abstract: The aim of this study is to evaluate the effect of an aerobic training program on the development of social maturity of non-athlete male students. The research method is semi-experimental. Sixty subjects participated in this study, and were assigned to two groups (test and control). Then by applying a training program (in test group) for a period of 12 weeks, the effect of training program on social maturity was studied. To collect data, a questionnaire which is based on Rao's Social Maturity Scale was distributed before and after the course. The results showed that social maturity rate of the participants was significantly different before and after the training program. Also, it was found that there was a significant difference between control and test groups after training program. A specific sport program can be effective in raising the social maturity level and its triple dimensions.
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1. Introduction

Physical education, as an important part of education system, has played as indispensable role in the physical, mental and social maturity of the students. It was reemphasized in National Summit on Physical Education, held at 24 January 2005 (London), during the UN International Year of Sport and Physical Education. The Summit brought together for the first time, leading experts from a range of disciplines and fields of study – professors, academics and practitioners in physical education – to debate the role of and need for physical education. Some issues regarding physical education including "the importance of physical competence as a means of enhancing self esteem, empowerment and social inclusion", and "the role of physical education as a positive learning experience for physical activity and prevention of obesity and promotion of healthy life styles" were taken into consideration (Declaration from the National Summit on Physical Education, 2005).

In the present time, universities have devoted as much attention to the students' health and physical care as they spend on their educational and academic growth (Bucher & Wuest, 1992). Accordingly, instructors and the teachers of physical education have effective roles in achieving the mentioned goals, and by adopting proper methods in teaching physical education courses can help students attain the intended aims and bring them health and a high spirit together with a growth in their personal, emotional and cultural maturity. So, we have intended to evaluate the effects of physical exercise on the growth and social maturity of non-athlete students in this study.

Social maturity and physical activity

According to Thomanna & Cartera (2008) social maturity is a remarkable pattern of manners that the people should be able to achieve. A desirable level of such manners as sociability, a sense of responsibility, flexibility and being domineering with the intention of gaining positive values and feelings are intended in this sense. Recent research (e.g. Zimmerman, 2004; Castelli et al., 2007; Peterson et al., 2007) has proved that the growth of social features of people (independence, competitiveness, art and sporting activities) are the result of gaining experiences through interacting with the family, peers, friends and the cultural milieu of society.

Nowadays, social maturity has been so widely considered by most experts that it has led them to the belief that a person's social growth and maturity form the basis of his/her whole life. Thus, the fundamentals of one's social life are compatibility with others, being in agreement with them, and somehow trying to meet their needs and expectations (Shariatmadari, 1993). As a result, we can say that the growing tendency of people, specially the youth, towards sporting activities has come to be an indispensable must (Noorbakhsh, 2004). In this regard, sports physiologists believe that taking sporting activities and exercise are necessarily important in providing and fortifying social skills and social maturity (Castelli et al., 2007; Tomporowski et

al., 2008). Moreover, sports sociologists maintain that taking exercise is very important in forming personal bearings of the people (Noorbakhsh, 2004). Because of this, universities, based on their organizational responsibilities, have also exercised much attention to advancing participation in physical exercise and sports, so as to provide the students with conditions of all-inclusive growth, especially skills of life. Hence, experts in the field of physical education have done comprehensive research regarding this important fact (Hardman, 2008).

Many studies (e.g. Alleyne, 2003; Noorbakhsh, 2004; Castelli et al., 2007) have been conducted regarding physical exercise and its role in improving the mental health and well-being of the people. Considering the background of such studies, it is shown that those who continually take exercise have a better emotional balance and do better in sporting situations. Such studies also show that the youth taking part in sporting activities have experienced a positive growth in such aspects as:

- Physical well-being and proper way of life;
- Self-confidence and self-esteem;
- Making motor skills;
- Making social skills;
- Sports ethics and philanthropy;
- Having high spirits;
- Increased motives; and
- Increased social maturity.

Also, results from some studies propose that taking part in sports activities like games and collective plays causes cognitive as well as intellectual development of the people (Bjorklund & Brown, 1998; InstructorWeb, 2002). According to InstructorWeb (2002), "the relation between regular physical activity and intellectual development is so clearly established that in today's educational world, most school jurisdictions have made physical education mandatory for all students". In a study on people of 12 to 40 years of age, Brown (1967) has proved in that those taking exercise (isometric meditative ones) had a more developed social maturity than those not taking such courses (non-exercise ES=0.08;exercise ES=0.86). Dwyer and colleagues (2001), through a study on children of 7 to 15 years of age, showed that there was a close relation between physical health and social maturity. The study of Castelli et al. (2007), on 259 children 3 to 5, has also proved the same results.

From what has been already said, it can be said that by providing the students with different fields of sports and encouraging them towards taking them, it is tried to, in addition to physical provision of the students, make for their personality growth in social aspects. Therefore, evaluating the maturity level of non-athlete students besides advancing the knowledge of this field can result in actual outcomes which can be used by those involved in planning and managing physical exercise.

Aim of the study

The main intention of this study was to conduct a special aerobic plan and measure its effects on the growth of social maturity of the non-athlete students. Accordingly, the study revolves around two main problems namely "what level of social maturity and its triple dimensions do the non-athlete students have?" and "how effective can an aerobic exercise plan be in raising the social maturity of the students?".

2. Material and Methods

The present study was conducted in a semi-experimental way. In this method, an exercise plan was tried as the independent variable and by considering the control and test groups, the probable effect of this training plan on social maturity and its triple dimensions (social competence, personal competence, and interpersonal competence) of the non-athlete students was evaluated. For this purpose, two groups of control and test were selected who filled out the questionnaires before and after the plan.

The statistical community of this study contained all the male students of Islamic Azad University, Hamedan Branch, 60 ones of whom voluntarily took part in this study forming the two groups of control and test. Thus, the subjects were:

1. 30 non-athlete men (control group)
2. 30 non-athlete men (test group)

In order to gather the necessary data and information, the questionnaire of Rao's Social Maturity Scale was used. The original version of this scale has 90 items which is in the form of a 4-option range (quite agree, agree, quite disagree, disagree). For the mentioned scale, the grades 1, 2, 3, and 4 were assigned, respectively, with 10 as the lowest and 40 as the highest value of the social maturity in the measured scale. In addition to being measured in general, social maturity was also measured in its 3 dimensions of personal, social and interpersonal competence.

After selecting the subjects and getting their written agreement for taking part in all the process of the experiment, the questionnaire was handed to them before the exercise plan. The maximum aerobic capacity of the subjects was measured based on *Rockport Aerobic Test*.

Then, the subjects took a 12-week exercise course including walking, jogging and endurance running. Each week was devoted to 3 sessions, each for 20 minutes at the beginning extending to 45 minutes in the last sessions. All the subjects started with maximum 50 percent of their heartbeat which increased

to 80 percent in the 12th week (Table 1). Before starting the program, the subjects took a 10-minute warm-up session doing light and stretching exercise, they also had a 10-minute stretching exercise at the end of each session to cool down.

At the end of 12th session, the questionnaires were again distributed and gathered after completing. Descriptive and inferential statistic methods were used for analyzing and evaluating the rough data so achieved. The descriptive approach was used for describing the features of the data taken from the control and test groups, and inferential one was applied for testing the presumptions of the study.

Table 1: The planned exercise program

Weeks	*Time devoted in minute*	*Exercise*	*Maximum heart rate*
1	15-20	Jogging	50%
2	20	Jogging	50-55%
3	20-25	Jogging	55%
4	25	Jogging	55-60%
5	25-30	Jogging	60%
6	30	Jogging	65%
7	30-35	Jogging	60-65%
8	35	Game	-
9	35-40	Jogging	65-70%
10	40	Jogging	70%
11	40-45	Jogging	75%
12	45	Jogging	80%

To evaluate the effects of the program on the subjects and find out about the difference between the two groups of test and control, all the statistical tests were done and analyzed using SPSS software.

3. Results

The calculated statistical data for social maturity and its triple dimensions are given in the Table 2. These data include the number of subjects, standard deviation, and mean achieved for each group of data before and after the program. As is clear the number of the subjects in each group was 30 ones.

Table 2: Statistical descriptives for social maturity and its triple dimensions in study groups

(Mean±SD)

Groups		**N**	**Personal Competence**	**Interpersonal Competence**	**Social Competence**	**Social maturity**
Control	Pre-test	30	26.20±4.93	28.40±2.66	24.26±2.57	78.86±6.81
	Post-test	30	25.96±4.64	28.03±2.63	24.00±2.61	78.00±6.71
Test	Pre-test	30	25.93±2.97	28.43±2.04	25.60±2.9	79.96±3.83
	Post-test	30	29.20±1.84	29.90±2.12	27.00±2.39	86.10±4.75

The results of covariance (Table 3) show that there was no significant difference in the personal competence between the test and control groups before the exercise program, but after the program there appeared a meaningful difference between the groups (p=0.001). The results from the T test also affirm these findings, and show that the test group has received a significant increase in their personal competence after the course.

Table 3: Results of covariance for personal competence in two groups

Personal competence		*Sum of Squares*	*df*	*Mean Square*	*F*	*Sig.*
Pre-test	**Between Groups**	1.067	1	1.067	.064	.801
	Within Groups	962.667	58	16.598		
	Total	963.733	59			
Post-test	**Between Groups**	156.817	1	156.817	12.567	.001
	Within Groups	723.767	58	12.479		
	Total	880.583	59			

In table 4 the result of interpersonal competence are presented which show the effects of exercise on the increase of interpersonal competence level, that is, such figures in the test group, at the end of the program, and in comparision with those of the control group before and after the program show a meaningful difference (p=0.004).

Table 4: Results of covariance for interpersonal competence in two groups

Interpersonal competence		*Sum of Squares*	*df*	*Mean Square*	*F*	*Sig.*
Pre-test	**Between Groups**	1.667	1	1.667	.003	.957
	Within Groups	326.567	58	5.630		
	Total	326.583	59			
Post-test	**Between Groups**	52.267	1	52.267	9.140	.004
	Within Groups	331.667	58	5.718		
	Total	383.933	59			

Table 5 presents the figures for social competence among different groups of the research. As can be seen, these figures for both test and control groups are slightly different but the difference is not meaningful before the program (p=0.060). Here also, as in other cases, the effect of taking exercise is tangible, that is, we observe an increase in social competence in the test group after the program, (p=0.000).

It is notable that a difference there appears between the test and control groups in social competence after the program.

Table 5: Results of covariance for social competence in two groups

Social competence		*Sum of Squares*	*df*	*Mean Square*	*F*	*Sig.*
Pre-test	**Between Groups**	26.667	1	26.667	3.691	.060
	Within Groups	419.067	58	7.225		
	Total	445.733	59			
Post-test	**Between Groups**	135.000	1	135.000	21.511	.000
	Within Groups	364.000	58	6.276		
	Total	499.000	59			

Results from analyzing covariance are given in Table 6 below. As we can see (see also Figure 1), the exercise plan has had a meaningful effect on increasing the students' social maturity (p= 0.000).

Table 6: Results of covariance for social maturity in two groups

Social maturity		*Sum of Squares*	*df*	*Mean Square*	*F*	*Sig.*
Pre-test	**Between Groups**	18.150	1	18.150	0.593	.444
	Within Groups	1774.433	58	30.594		
	Total	1792.583	59			
Post-test	**Between Groups**	984.150	1	984.150	29.083	.000
	Within Groups	1962.700	58	33.840		
	Total	2964.850	59			

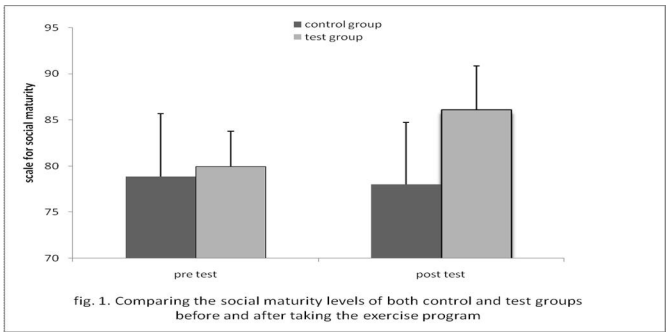

fig. 1. Comparing the social maturity levels of both control and test groups before and after taking the exercise program

4. Discussions

As we see from the results, the figures for social maturity and its triple dimension have not greatly changed before and after the exercise program in the control group. But those of test group showed a great difference before and after the test. This difference was statistically meaningful. Also, before taking the test, there was no meaningful difference between the control and test groups regarding their social maturity; but after conducting this study there appeared a meaningful difference between them in their social maturity and its triple dimensions. This means that the aerobic program has had a positive effect on increasing the level of subjects' social maturity in the test group. This finding is compatible with previous studies. In a word, related studies have also pointed out this. Among such studies is Vaez-Moosavi (2001) who observed in his study that features like physical health, sociability, philanthropy, creative conduct, and job satisfaction were better developed in athletes than in non-athlete individuals. Additionally, Bloorchian (1992) concluded that sport and recreational trips have a positive impact on people's sense of responsibility, health, cooperation, self-confidence and being charitable.

Also, Brown (1967) has proved that those taking exercise had more developed social maturity than those not taking such courses. Heckel and co-workers (1977) observed in their study that sports could help people be more cooperative, increase their capacities and avoid selfishness and being domineering. Behar & Stephens (1978) in their studies found that people's relations and family relations could get better by taking exercise and such people had not shown criminal tendencies. Furthermore, Schoberberger et al. (1981) in Germany found out that sport could greatly increase people's independence and positive sense of self-confidence. It also changed, somehow, their consideration of sickness and some other mental variables. Vyhnalek & Vavrova (1990), conducting a study in Czech, concluded that taking exercise caused a better understanding of one's personality, improvement of independence, better adaptability with illness and increase of self-confidence in the patients. Ewing (1997) showed in his study that parents could cause their children's social growth by providing them with sporting opportunities. Instructors can help subjects' social growth by giving them positive feedback. Besides, by giving good instruction, using encouraging speech, and having a logical viewpoint in correcting errors, teachers can ensure children's continuation of exercise and training. In addition to increasing social qualification, taking exercise can lead to the growth of other forms of self-confidence among the youth.

Based on the present study and those of others aforementioned we can conclude that sport and exercise programs have telling effects on mental and physical health, self-confidence and social maturity. Consequently, the exercise program used in the study has successfully increased the social maturity level of the male student. Also, in the three dimensions of social maturity, exercise has been meaningfully effective. In general, the results achieved from this study proved that the 12-week exercise program has had a tangible effect on the social maturity and its triple dimensions (social competence, personal competence, interpersonal competence) of the students in the test group as compared to those of the control group. As closing remark, the present research findings suggest that systematic exercise programs may actually enhance the development of social maturity which is of critical importance for coping with challenges encountered both in academic period and throughout the lifespan. A glance at the studies conducted in this field proves that their results are in agreement of those of this study.

As for the future, it is suggested that this study is comparatively performed by gender. For better generalization, it is advised that such studies are applied for larger scale populations.

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Nature and Domain of Islamic Education

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Abstract: There are three attitudes toward the Islamic education. In the first attitude, the Islamic education is considered as a descriptive system which can be achieved through studying the works of Islamic scholars. In the second attitude, it is an inferential system based on which replies can be provided for all educational problems using the religious texts. In the third attitude, the Islamic education is considered as an inferential-institutional system in which some problems and discussions related to education are extracted from the Islamic texts and some other items are obtained based on empirical methods and religious presuppositions. With a short glance at nature and history of the Islamic education as an academic discipline, this writing tries to survey its research methods and its achievements. (Bagheri, Khosro, 2000)
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Keywords: Islamic education (existing attitudes in the Islamic education), Islamic education history, research methods in the Islamic education, the relationship of the Islamic education with other educational sciences disciplines

1. Introduction

The Islamic education is different from what is customary in the current educational sciences. The cause is that the contemporary educational sciences, being in the domain of empirical sciences, have diverged from the religions and ideologies and have sought for rendering an empirical knowledge. Even in the domain of aims and achievements of education, this independence from religions and ideologies continues because investigation of such affairs is dominantly done in the field of the philosophy of education.(Ashraf, Ali, 1977-1989)

However in our land and also in some Islamic countries, because of influence and dominance of Islam on thought and life of the people, talking of the relation between Islam and education is of great importance. In a way parallel to formation of the educational sciences in the contemporary history, there has also been an attempt to organize the old heritage of Islamic thought and culture, so that a system of educational sciences can be formed based on this great heritage, as a response to the educational needs of the countries. Talking of the Islamic educational more or less implies confronting such a challenge.

With a glance at the nature and history of the Islamic education as an academic discipline, this writing tries to survey its research methods and achievements.(Bagheri, Khosro,2000)

2. Definition

To define the Islamic education as an academic discipline it can be said that the Islamic education implies a thinking system directly or indirectly originating from the Islamic texts and it contains knowledge of the phenomena and the existing relations in education domain, their favorite forms and the way of changing them.

Clarification of the definition involves explanation of some basic concepts which are used in the field. First, the Islamic education is considered a «thinking system» because it implies some kind of knowledge: knowledge of what goes on or must go on the practical domain of education. (Rafeyi, Behruz,1999)

The second and third concepts are «direct and indirect» origination of this knowledge from «the Islamic religious texts». The latter concept, i.e. the Islamic religious texts means the formal Islamic texts including the Quran and religious speeches (Hadis). It is worth mentioning that the practical living way of religious leaders has generally been mentioned among the religious speeches and texts.

But what is meant by «direct and indirect»? This concept can be assumed with two meanings. In the first meaning, «direct» implies that some issues are extracted from the Quran and Hadis and «indirect» refers to the issues existing in the Islamic culture and Muslim scholars' works and they are described «indirect» because they are more or less inspired by the formal Islamic texts.(Javadi Amoli, Abdollah.1993)

The second meaning of the word «direct» is based on a religious presupposition and refers to the fact that the Islamic religious texts contain replies to all questions. This kind of comprehensive study of religion is expressed with different degrees of strength. So you can talk of the two strong and weak forms in this kind of study of religion. In the strong form, the comprehensive study of religion implies that replies to all general and specific questions related to different

fields including education exist in the religious texts and if some details of affairs have not been mentioned in the Islamic texts, there are principles which can be used as a basis for bifurcation. Bifurcation means an attempt to go from the principles or general rules to the details in a way that those principles can cover the specific affairs. Conversely, it can be said that it involves putting the specific affairs under the coverage of one or a few principles.

In the weak form of comprehensive study of religion, an «option zone» is assumed in which the jurisconsult can determine the commandment for some affairs. The «option zone» is related to the allowable affairs. In other words, the behaviors and cases existing in this zone are covered by canon commandments because they are considered religiously allowable, however the jurisconsult, with the permission which he has from the god and with regard to the interests of the society, can make these allowable affairs necessitous or unlawful when necessary. Regarding this point, the option zone is both under the commandment of both the canon and the jurisconsult. (Sadr, Mohammad-Bagher,1979)

The word «indirect» is based on a different religious presupposition and shows that the Islamic religious texts is not responsible for answering all the questions but in a selective way they cover the problems related to different fields. In other words, in some fields the general and subsidiary problems have been noted and replied but in others just the general and essential problems have been noted. In some fields, there have been just some references and some fields have not been referred to.

Regarding the points, the meaning of «indirect» refers to the fact that the knowledge of Islamic education is a result of expansion of general discussions mentioned in the religious texts. One kind of these expansion cases can be paving the way for empirical researches in the field of education based on the general and essential teachings of Islam on human.

The forth concept is the phenomena and existing relations in the practical domain of education. This concept includes the collection of phenomena and the relations among them in the domain of education. For example, teacher and student are two phenomena in the domain and one of the relations between them is teaching. Obviously the defective forms of the affairs are also covered implicationally by this concept.

The fifth concept is «the favorite forms» of the phenomena and the relations. This Concept includes the aims and norms toward which the phenomena and relations should move.

The sixth and last concept is «the way of changing» the phenomena and relations and giving them a favorite form. This concept includes the principles and methods of education. In other words the way of changing includes the rules of general procedures (principles) and details (method) for changing.

3. Three Attitudes toward the Islamic education

Based on what was mentioned before, we can say that the Islamic education as a thinking system in the practical domain of education can have at least three attitudes which do not necessarily come together:

1. The Islamic education as a descriptive system: In this case the Islamic education is achieved by studying the works of the Islamic scientists. As these works have generally organized and written by the authors themselves, the aim can be achieved through a more or less simple description of the works and sometimes through subsidiary analysis and classification.

2. The Islamic education as an inferential system: in this view, replies can be found for all educational problems in the Islamic religious texts. Some kind of comprehensive study of religion is a presupposition for this attitude. Accordingly, regarding the educational problems, replies can be found indirectly from the Islamic texts. In the strong form of this attitude, it is assumed that replies to all small and big problems of education exist in the Islamic texts and they should be achieved through surveying the texts and if some replies do not exist directly, they can be found through defining the point that to which principle the problem or the behavior belongs. In the weak form of the attitude, some cases which at the first step are reckoned religiously allowable, may receive a commandment by the jurisconsultant as being necessitous or forbidden.

3. The Islamic education as an inferential-constitutional system: In this view, because some problems and discussions related to the education have mentioned the Islamic texts, the extraction and inference of these issues represent the inferential aspect of the attitude however some issues have not been paid attention in the Islamic texts and their replies can not be found through bifurcation because these issues are of explorative kind. In other words, it includes clarification of an external entity not expression of a jurisconsultant's commandment on a certain case or behavior. For example these problems can be considered: What mechanism do learning and learning defects occur with? Replying such questions requires establishment of knowledge on the phenomena. Here we confront an empirical science but presuppositions of this empirical science can be obtained from the Islamic texts. These presuppositions including the characteristics of the subject (human) and the studying method are among those inferential issues. However, reliance on these presuppositions and suggesting hypotheses and testing them and achieving the new findings relate to the constitutional aspect of the attitude.

4. History

Some courses and steps have been mentioned for the Islamic education including the following classification: The first course covers the first half of the first Hejri century and was accompanied by emergence and development of literacy in the form of reading and writing the Quran sentences. The second course which consists of two sections (50-350 and 351-600) includes the familiarity with sciences and technics of other nations. The third course which lasted from the 7th century (Attack of Mongols) to the 13th century was a recession period for the Islamic education. The fourth course covering the 13th and 14th centuries includes contact of the Islamic education with increasing changes of new education.

This classification refers more to the practical procedure of the Islamic education than to the Islamic education as a branch of knowledge. The history of the Islamic education in this meaning can be date back to the 2nd and 3rd centuries, regarding the works like «rites of the learners» by Mohammad Ebn Sahnun (Death in 256). Afterwards, the opinions of scholars in the field of ethics, philosophy and theosophy like Farabi, Ebn Maskuyeh, Avicenna, Ghazzali and Mowlavi Rumi can be considered as chains of the Islamic thought in the field of education.(Sharifi, Hadi,1977)

But if you consider the Islamic education as a distinct academic discipline, its history will be reckoned contemporary. Since about half a century ago, some Muslim thinkers like Mohammad Ghotb have started writing in the field of the Islamic education. But a turning point was the establishment of a global congress of the Islamic countries including Iran in Mecca in 1997. In this congress, about 330 Muslim scientists took part and studied the hard situation of education in the Islamic countries and the necessity for compiling the Islamic thoughts on the education. Since then four other congresses have been established and they have paid attention to more practical and more technical issues of education. The second congress was in Islamabad, Pakistan, in 1980 titled «Teacher education» and the fifth one was in Rabita, Morocco, for evaluating the previous congresses.

In Iran after the Islamic revolution, the Islamic education was formally established as an academic discipline at the Teacher Training University at MA level in 1990.

5. Research Methods

Based on the nature or the identity of the Islamic education, the research methods will be different. Parallel to the mentioned attitudes toward the Islamic education, there are different research methods in this field.

If you assume the first attitude, i. e. the Islamic education as a descriptive system, the appropriate research method will be the method of description and content analysis. Accordingly, the researcher seeks for rendering a description of education through studying their written works. When such works have been organized well, a simple description will be suitable. Otherwise it is necessary to analyze the content of different works of a scholar and classify them based on the issues and discussions of education in an attempt to describe his viewpoint in this field.

Regarding the second attitude, i.e. the Islamic education as an inferential system, the research method will be juridical and expository inference, which has long been used by the jurisconsultants and expositors. In this method using some rules and measures, scholars' try to reach commandments based on the religious texts and make decisions for different situations. For example «appearance rationality» is a rule implying that the appearance of the discourse mentioned in the religious text own validity and the meaning which is achieved based on the appearance is notable (however it is not always the word appearance but it may be the specific kinds of appearance like a customary one). Such rules provide the possibility for inferring the canon commandments or understanding the religious texts.(Kuhn, Thomas ,1970)

Using this method as the research method in the Islamic education means that the researchers, in order to find the answers to the educational questions with the aid of the mentioned principles and rules, will study the religious texts. However in the two forms of this attitude there will be some differences in the method. In the first form, the answer or the issue itself is sought for in the religious texts and when necessary, the researcher tries to relate the subsidiary aspects to the general principles and then determine the commandment. In the second form, he involves in the social facts of the time in determining the commandment.

Finally, regarding the third attitude, i.e. the Islamic education as an inferential-constitutional system, the research method will have two dimensions. In the first dimension, the expository and inferential method is used for understanding the religious texts and extracting issues as presuppositions of the empirical research (as well as the issues extracted from the religious texts in the field of education system contents like the aims or principles). In the second dimension, it is necessary to use the methods of empirical research for establishing and providing new sciences in the field of education. Achievement to hypotheses inspired by the mentioned presuppositions about the educational phenomena will not be possible without following the external evidences. The empirical method has been used in the broad sense of the word, i.e. surveying the external evidences for or against the hypothesis. So what has been mentioned as a opposition between the

qualitative and quantitative methods can not represent here because both kinds are subsections of the empirical method in a broad sense.(Ezzati, Abolfazl,1977)

6. Relationship with other educational sciences

The kind of relationship of the Islamic education with other educational sciences depends on which attitude we are considering from among the three mentioned ones.

If the first attitude, i.e. the Islamic education as a descriptive system is assumed, its place will be in the history and philosophy of education field. In other words, studying the Muslim thinkers' works gives the Islamic education discipline historic and philosophic color, so it must be in line with other historical and philosophical views.

When the second attitude, i.e. the Islamic education as an inferential system is assumed, its relation with other educational disciplines is of kind of independence and peculiarity. In other words, in this view the Islamic education does not need the contemporary educational sciences because the answer to every question can be found in the religious texts and in addition there is no doubt on the correctness of the answers but what is achieved in the educational sciences can be false. Peculiarity originates from this point: the speech whose truth is definite will be different from the speech whose being false is always possible.

This kind of relation is observed in the first form of this attitude but in the second form, i.e. belief in the option zone, a relation of the kind of complementary can exist. In other words, those cases which are in the option zone and in the first step are considered allowable, after the jurisconsultant gets information on the achievements of the educational sciences, may receive the commandment of necessitous or unlawful. As the achievements of the educational sciences help the jurisconsultant in specification of the appropriate aspects, there is a complementary relationship here.

Finally, in the third attitude, i.e. the education as an inferential-constitutional system, the relation will be a paradigmatic competition relation. When the Islamic education, with emphasis on the presuppositions achieved from the Islamic texts, goes toward the empirical research, its relation with the contemporary educational sciences will be like the relation between two scientific theories which exist in two different domains. According to Thomas Kuhn, there is an essential competition between the two theories related to two scientific paradigms in such a way that the experiences of a paradigm when are located in another paradigm, will need a different exposition appropriate to the new paradigm. So in this attitude the Islamic education will hold such a relation with the educational sciences. In other words, the findings and experiences in other educational disciplines in the framework of the Islamic paradigm should be interpreted with regard to its specific concepts. This relation will create a dynamic competition between the Islamic education and the educational sciences which will result in the development in the Islamic education.(Mesbah, Mohammad-Taghi,1968)

Just another point should be referred to and it is that incommensurability of the scientific paradigms according to Kuhn needs a review. This review is necessary because the incommensurability relation leaves no place for the overlap of scientific theories. This kind of relation puts the scientific attempt under the threat of relativism because every paradigm does a meaningful and valid activity just in its own framework and this prohibits dialogue between different theories of the scientific paradigms. Kuhn considers the benefit of these theories in solving the problems as a measure to select or reject them. However regarding the internal frameworks of the scientific paradigms he does not believe in a relation and overlap between them. The necessary review in this review is that the possibility of overlap will not be rejected rather this possibility should be reckoned that some things in common and overlaps in the methodology domain may emerge with the findings.

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EAPHRN: Energy-Aware PEGASIS-Based Hierarchal Routing Protocol for Wireless Sensor Networks

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Abstract. Power management is considered as one of the most critical researching issues in the area of wireless sensor networks (WSN), as it plays the main role in increasing the sensor nodes life time. This paper proposes a new hierarchical routing protocol for stationary wireless sensor networks, called EAPHRN Energy-Aware PEGASIS-Based Hierarchal Routing Protocol for Wireless Sensor Networks (EAPHRN). The proposed scheme attempts to increase both the lifetime and the throughput of the wireless sensor network. The efficiency of proposed protocol is evaluated. The simulation results showed that the EAPHRN protocol can solve the main problems in PEGASIS since it uses a new chain construction algorithm that is completely different that the PEGASIS and is more efficient. It also uses a new chain leader election method that plays a very critical role in the energy saving.
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1. Introduction

A wireless sensor network (WSN) consists of numerous tiny autonomous sensing nodes that are deployed across a wide geographical area. Routing in WSN differs according to the type of its network structure. Two basic network structures are used: Hierarchal structure and Flat structure. The Hierarchal routing is the routing that operates in the hierarchal structures of WSNs. The main goal of the hierarchal routing protocols is to save the energy of sensor nodes as much as possible, and hence it prolongs the WSN lifetime. To design an energy aware hierarchal routing protocol, two main issues must be considered: the network model and the leader election method. The network model is considered as the environment of routing. Designing an energy-aware network model requires the routing protocol to be an energy-aware protocol. A good network model is one that guarantees that the routing paths will be as short as possible and that the sensor nodes will communicate with adjacent neighbors; thus they do not need to increase the transmission power which is considered to be one of the most important factors in the node's energy consumption [11, 12, 13].

As previously mentioned, in the hierarchal routing there are elected nodes (one or more) that are acting as a local base station inside a cluster or a chain, those nodes collect the data gathered by sensor nodes and combine it together, then they send it to the BS or to other further regions inside the WSN. It is very important to change the leader(s) frequently, since being a leader means a high rate of power consumption and the reason is clear. For this, it is important to have a leader election method, which attempts to elect a new leader with good conditions to do the job. Choosing the wrong leader means a fast depleting for it and a decreasing for the total lifetime of the WSN, while choosing a leader with reasonable conditions means prolonging the network's life time.

In this paper, we propose a new hierarchical routing protocol for stationary wireless sensor networks, called Energy-Aware PEGASIS-Based Hierarchal Routing Protocol for Wireless Sensor Networks (EAPHRN) and compare it with PEGASIS scheme.

The rest of the paper is organized as follows. Section 2 provides a brief overview of the related work. Section 3 explains the system and energy models. In section 4 we present the proposed EAPHRN. In section 5 we evaluate and analyze the performance of EAPHRN. Finally, we draw the conclusion and future work in section 6.

2. Related Work

In this section, we present a review of some hierarchal routing protocols that attempt to prolong the network's life. LEACH protocol [2, 10] is one of the classical hierarchal routing protocols. It uses a clustered network topology that has a number of groups. Each group contains sensor nodes that share some geographical parameters. All nodes within one cluster communicate with an elected cluster head node, which in turn communicates directly with the

BS on behalf of the nodes within the cluster. The election of a new cluster leader is performed at each round and it is based on the randomization selection.

PEGASIS [1] is another classical hierarchal routing protocol that is examined in this study. As mentioned before, PEGASIS forms a chain covering all nodes in the network using a greedy algorithm so that each node communicates with only the neighboring nodes. In each round of communication, a randomly selected node in the chain takes turns to transmit the aggregated information to the BS to save the energy. Furthermore, the elimination of the cluster setup phase, like the one employed in LEACH, allows considerable energy saving. However, the communication delay can be large due to the long single chain and the possibility of having large distances between neighbors inside the chain.

CHIRON [3] is a very new hierarchal routing protocol that has the benefits of both clustering and chaining. In CHIRON, the geographical area is divided into zones using the BeemStar technique [4]. In each zone a chain is formed covering all nodes in the zone using a greedy algorithm. The farthest node(s) within each chain is (are) elected as chain leader(s) for the first round. After the zoning phase is completed, another chaining that covers all chain leaders starting from the farthest node in the network is performed. Finally the closest leader to be BS is elected to be the leader of leaders. CHIRON changes its leaders for each round based on the maximum residual energy of nodes within each zone, which means that the second layer of chaining must be constructed for each round.

3. The System and Energy Models

3.1 The System Model and Assumptions

The system model of the wireless sensor network is considered to be consisting of one sink node (Base Station) and a large number of immobile sensor nodes. The sensor nodes are uniformly deployed over the target area to continuously monitor the environment. Assumptions about the sensor nodes and the underlying network are as follows:

1- There is a BS located far away from the geographical area where sensors are randomly scattered. The sensors and the BS are all stationary after deployment. Figure 1 below illustrates an example of such network topology.
2- All nodes are homogeneous and have the same capabilities.
3- Each node is under the coverage of the BS as well as all under other nodes coverage.
4- The nodes can vary in the amount of transmission power depending on the distance to the receiver. Each node can reach the BS directly.
5- Data are periodically transmitted from the sensor node to the remote BS.
6- The links are bi-directional.
7- All packets that are transmitted are of the same size.

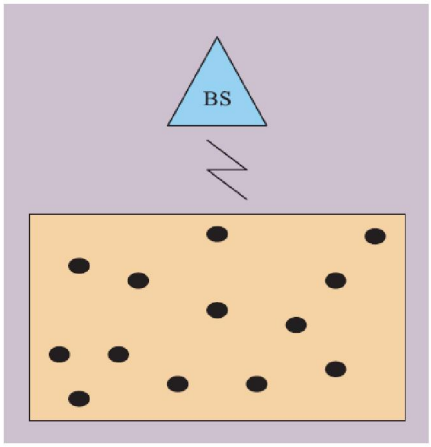

Figure 1: Typical wireless sensor network topology

3.2 Energy Model

We adopt the same radio model as stated in [1, 5] with *Eelec*=50*nJ/bit* as the energy being dissipated to run the transmitter or receiver and $\in$amp = 100 pJ/bit/m2 for the transmitter amplifier. The radios have power control and can expend the minimum required energy to reach the intended recipients. The radios can be turned off to avoid receiving unintended transmissions. The equations used to calculate transmission costs and receiving costs for a *k*-bit message and a distance *d* are shown below.

For transmitting:

$$ETx(k, d) = ETx\text{–}elec(k) + ETx\text{–}amp(k,d) \quad (1)$$

$$ETx(k, d) = Eelec*k + \in amp * k* d^2 \quad (2)$$

For receiving:

$$ERx(k) = ERx\text{-}elec(k) \quad (3)$$

$$ERx(k) = Eelec*k \quad (4)$$

The energy of data aggregation (EDA) is 5 nJ/bit/signal.

4. The Proposed EAPHRN

The goal of the proposed protocol is to design a hierarchal chain-based routing protocol that

attempts to be as optimal as possible in terms of power consumption. The idea is to find a low cost chain that covers all nodes of the network as in the PEGASIS [1] protocol. As mentioned before, the PEGASIS uses a greedy algorithm to build the chain starting from the farthest node in the network until all nodes are connected .This chain is appropriate because the greedy algorithm tries always to find the next closest neighbor to connect to. However, in large networks, the greedy algorithm causes serious problems and leads to the problem of long chain [3, 4, 6, 7]. To have a clear understanding of this problem, consider the following scenario illustrated in figure 2 below:

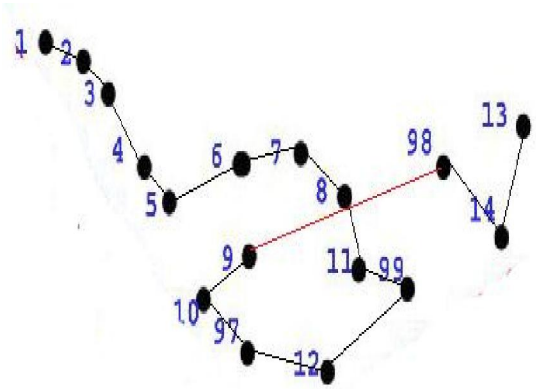

Figure 2 The Long Chain problem

The greedy algorithm starts operating from node labeled "1",and the process continues by connecting the next closest neighbor that is not included in the chain until the node labeled "9" is connected . After that, this node must connect to the next closest node but the next one, node 98, will be extremely far because the close neighbors of node 9 have already been connected to the chain. In this situation both nodes "9" and "98" will consume a high rate of energy while keeping other nodes operated in very good conditions. Furthermore, when nodes 9 and 98 die. The nodes directly connected to them, which are node 10 and node 14 will be operating in the same bad conditions and will be expected to die sooner than the other nodes and so on.

To solve this problem, the new chain construction algorithm in the proposed EAPHRN makes an enhancement over the greedy algorithm, so that the new algorithm does not connect the "next closest node" .Instead, it connects a random node that is located not farther than a Distance Threshold (DT). In this situation the algorithm will connect nodes as follows: "pickup a random node from a group of potential nodes, all are within a distance threshold DT. The DT should be a reasonable distance that can be applied to all nodes in the network so that nodes do not consume a high rate of energy. By using this algorithm, the chain connects nodes so that all distances between neighbors (edges) do not exceed a reasonable distance, and hence all nodes have fair energy consumption. Figure 3 below illustrates the result of applying this algorithm with the case presented in figure 2.

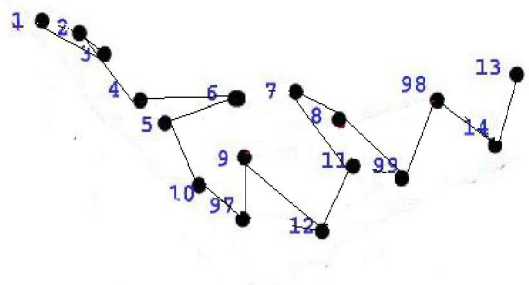

Figure 3 Solving the Long Chain problem

The procedure of EAPHRN is divided into two main phases: the chain setup phase and the leader election method phase.

A. Chain Setup Phase

Before the chain is constructed, the DT must be computed first. Both the BS and the sensor nodes do some contribution in the algorithm that computes DT. Each node must compute a Local DT (LDT), which is the average of distances between the node and the closest *n* nodes to it where *n* is a constant that is determined based on the number of all nodes in the WSN. The Equation of computing LDT is as the following:

$$LDT = \frac{\sum_{i=1}^{n} dst(i)}{n} \qquad (5)$$

Where *dst(i)* is the distance between the node that runs the computation and the closest i^{th} node to it.

After the LDT is computed, the node sends it to the BS as a low cost control message. When the BS gathers all LDTs from all nodes, it computes the DT and sends it to all nodes in the WSN to start forming the chain. The equation to compute the DT is as the following:

$$DT = \frac{\sum LDT}{m} \qquad (6)$$

Where, *m* is the number of nodes in the wireless sensor network.

After the DT is computed and sent to all nodes in the WSN, the chain formation is started at the farthest node from the BS. At each node , all neighbors that are within the range of DT distance are considered potentials to be the next connected node .One of those potentials is picked randomly and is connected to the chain , and the process continues at this connected node.

Finally, when the chain is formed, a one chain leader is elected, which will be the closest node to the BS in the initial stage of the operation. Each node starts sensing and forwarding the gathered data to the next node in the chain, which in turn fuses it with its own data and forwards it to the next node and so on. Once the chain leader receives the data, it makes the last step of aggregation and sends the data to the BS.

B. Leader Election

As in all hierarchal routing protocols, the leader must be changed frequently since it usually consumes the highest rate of battery energy [8]. Most hierarchal routing protocols change the leader of the chain/cluster at each round. The new leader election is most likely based on the maximum residual energy, as in the case in CHIRON [3], or it is based on a randomization election as in LEACH [2] and PEGASIS [1]. However, in the proposed routing protocol, the election will be based on both of those criteria. The goal of the leader election method is not to find an acceptable leader; rather it is to find an optimal leader using the same factor that causes the power consumption which is the distance between the sender and the receiver [11]. Since each node knows the distance between itself and the BS, and since each node can compute how much the residual energy will be consumed if it has been elected to be a leader using the energy consumption equation number (1), then the least ratio of energy consumption can be easily detected and the node with the least ratio of power consumption will definitely be the best leader for the next round. The equation of the energy consumption ratio is as the following:

$$Ratio = \frac{EnCons}{EnResidual} \times 100\% \qquad (7)$$

Where, *EnCons* is the amount of energy that is consumed if the node is elected as a leader, and *EnResidual* is the amount if residual energy in the node's battery.

This election method guarantees that no nodes with bad conditions will be elected at all to be leaders even for one round. It is run at every round and the same leader can still be operating for many continuous rounds, and will not be changed unless another node becomes in a better situation in terms of energy consumption ratio.

The main advantage of this proposed routing protocol is that it attempts to save the energy for each node as well as make a balance of energy consumption in the WSN. The chain construction algorithm attempts to distribute the distances (edges) between nodes in order to avoid the existence of large distances between the nodes in the chain like those which occur using the greedy algorithm in PEGASIS [1].The leader election method guarantees that the best leader node with the least energy consumption ration will be elected. This plays a significant role in energy consumption and prolonging the networks lifetime.

5. Performance Evaluations

5.1 Simulation Environment

The simulation was run on a computer system, and the tool that is used to evaluate the proposed protocol is Omnet++. Omnet++ is an extensible, modular, component-based C++ library and framework for building network simulators [9].

The simulation tool and the computer system parameters are illustrated in table 1.

Table 1 The simulator and the computer system parameters

Parameter	Value
Simulator	OmNeT++ v4.0
System model	HP Pavilion dv 2500 Notebook
CPU	Core2 Duo CPU 2.00GHz(2CPUs)
RAM	2550 MB RAM
Operating System	Windows Vista Business

5.2 The Simulation Environment Parameters

For the simulation, 100 nodes are randomly scattered over a 1000m × 1000m geographical region. The simulation was run with the new proposed protocol as well as the PEGASIS protocol. The simulation environment parameters are illustrated in table 2.

Table 2 The simulation environment parameters

Parameter	Value
Network size	1000m × 1000m
Base station location	(995,995)
Nodes	100
Initial Energy	0.5 J
Eelec	50 nJ/bit
EDA	5nJ/bit
Data packet size	2000 bits

5.3 The Simulation Operation

The simulation was run according to the following steps:

Step1: The PEGASIS [1] protocol was simulated. The key metric for the simulation was to compute the number of rounds during the lifetime of the network. When a node dies, the simulation notifies the user that a node has died and the number of rounds that were completed at that point is reinforced. The PEGASIS chain construction phase with the simulation scenario phase is illustrated in fig. 4.

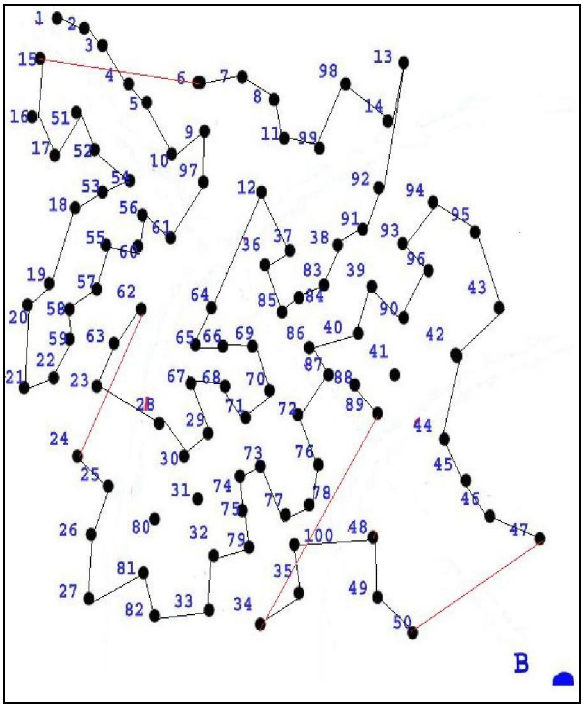

Figure 4. The PEGASIS chain construction phase with the simulation scenario

According to figure 4, the long chain problem of PEGASIS is obvious; there are many large distances, such as the distances between node 15 and 6, node 24 and 62, node 47 and 50, and node 34 and 89.

Step2: In this step, the proposed EAPHRN routing protocol was simulated. The same process that was done in step1 was applied. The LDT value was computed using the equation number (6),and the degree of *n* was 5 .The EAPHRN protocol's chain construction phase with the simulation scenario phase are illustrated in figure 5 below.

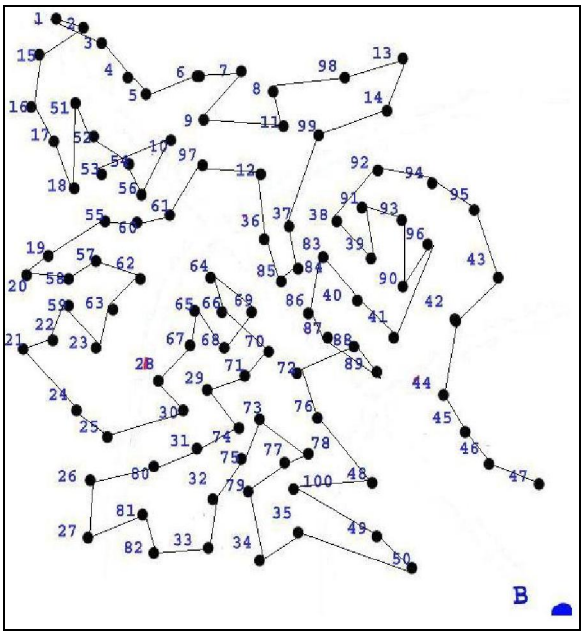

Figure 5. The EAPHRN protocol's chain construction phase with the simulation scenario

According to Figure 5 no large distances exist, like those that were found after applying the greed algorithm of PEGASIS, which implies that the proposed routing protocol will operate with reasonable rate of energy consumption.

5.4 Simulation Results

The goal of the simulation is to compute the number of rounds that each of the compared protocols can achieve against the number of dead nodes lost by the network when that number of rounds is achieved. This measurement proves the efficiency of a simulated protocol, in terms of power consumption.

For each protocol, the simulation was run and the number of achieved rounds was computed against different values of dead nodes as shown in table 3.

As shown in table 3, the efficiency analysis for results can be summarized as follow:

1- The proposed protocol (EAPHRN) is keeping node lifetime double times than PEGASIS.
2- For the network utilization in the proposed protocol (EAPHRN) the throughput is increased. There are no nodes died for the first 50 rounds, while in the PEGASIS the nodes started to die in the first of 14 rounds.

According to the simulation results, the proposed EAPHRN protocol is more energy efficient than PEGASIS as shown in fig. 6.

Table 3 Simulation results in terms of number of rounds against number of dead nodes

Percentage of dead nodes	Number of Rounds/PEGASIS	Number of Rounds/ EAPHRN
1%	14	49
10%	68	164
25%	116	232
50%	170	332
75%	216	416

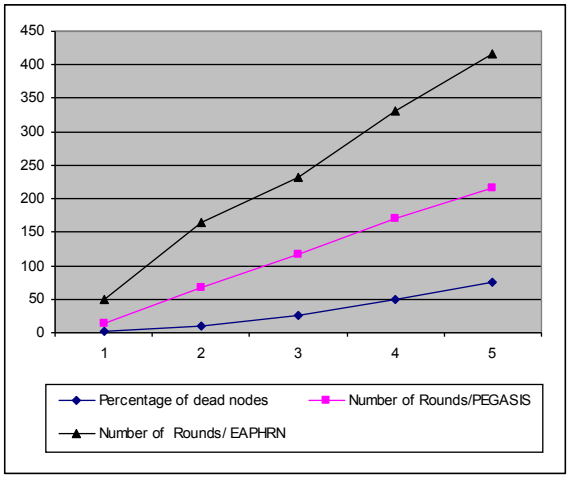

Figure 6. Column chart of the simulation comparison

6. Conclusion and Future Work

A new hierarchal routing protocol called Energy-Aware PEGASIS-Based Hierarchal Routing Protocol for Wireless Sensor Networks (EAPHRN) was proposed, which attempts to increase both the lifetime and the throughput of the wireless sensor network. We evaluate the efficiency of our proposed protocol and the simulation results showed that the EAPHRN protocol solved the main problems in PEGASIS, since it uses a new chain construction algorithm that is completely different that the PEGASIS one and is more efficient. Also it uses a new chain leader election method that plays a very critical role in the energy saving.

In the future works, EAPHRN will be compared with CHIRON [3] that is considered a new PEGASIS-based protocol.

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Effect of Forward Speed, Load and Cabin on Tractor Noise and the Health of Drivers

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Abstract: Noise has caused hearing problems to many drivers in the world. It has been investigated that 30 percent of Sweden's farmers suffer from hearing loss. Similar results to those from Sweden were found in a study conducted by University of Iowa in the United States, indicating that American farm workers are faced with the same noise problems in their daily work. A MF399 tractor without cabin and a Valtra 170 with open cabin were tested for noise level at the driver as well as the bystander ear for no load and loaded cases. Comparison were made between noise level in different forward speeds for each tractor with no cabin and open window cabin on driver's ear and bystander for no load and loaded cases. Also Comparison between no cabin and open cabin in each forward speed for no load and loaded tractor at driver's ear as well as at bystander. The noise level at 2.9 km/h for no load open cabin as well as for loaded no cabin and open cabin at driver's ear was significantly greatest among the related speed ranges. It looked like the 2.9 km/h speed with one exception was a threshold after which the SPL (Sound Pressure Level) dropped. For no load no cabin case at the driver ear with a mean value of 87.72 dB(A) no significant difference was observed at 1% level for speeds up to 3.7 km/h; but the SPL for 6.1 km/h was significantly higher. The permissible exposure time was calculated as 4 h/day and 1.9 h/day respectively. For no load open cabin case no significant difference in SPL between SPL at speeds 1.9, 2.2 and 6.1 km/h with a mean value 83.1 dB(A). However significant difference was observed between 2.9 km/h and 3.7 km/h speed. For loaded no cabin, no significant difference was observed between SPL of 1.9, 2.9 and 3.7 km/h with mean value 93.5 dB(A) and 1.1 h/day permissible exposure time. For loaded open cabin. No significant difference between SPL for 3.7 and 6.1 km/h with mean 89.2 dB(A) and permissible exposure time of 5.4 h/day. Also no significant difference between the SPL for 1.9 and 2.2 km/h with mean 83.7 dB(A). Bar graph for all exposure times are shown. The same comparisons were made at bystander's ear and between no cabin and open cabin in each forward speed.
[Mehrzad Payandeh, Mansoor Behroozi Lar, Jahangir Bagheri and Zahra Khodarahm Pour. Effect of Forward Speed, Load and Cabin on Tractor Noise and the Health of Drivers. Journal of American Science 2011;7(8):759-765]. (ISSN: 1545-1003). http://www.americanscience.org.

Keyword: sound level, noise measurements, tractors, cabin, driver's ear

1. Introduction

Noise or unwanted sound is one of the major sources of discomfort to the workers which affects human both psychologically and physically. It has caused hearing problems to many drivers in the world. Noise is measured in two ways for OECD tests (Anonymous, 2010): at the operator's ear and from a bystander position, and is measured in decibels [dB(A)]. But to the human ear, a noise that sounds twice (or half) as loud is actually measured at 10 dB(A). Sound measurement is made on the test track in two locations-at the driver's ear and in a location representing "bystander noise. The tests at the driver's ear are performed in several gears and under a number of conditions; but only the maximum level is reported. The "bystander sound" test is performed with the microphone located at 7.5 m from the centerline of the tractor which is accelerating from a lower speed to full speed in its top gear. The OECD procedure differs. The SAE/ASABE procedure measures sound in only one gear under different load conditions, whereas the OECD procedure measures sound in different gears between High Idle and Rated Engine speed (Larsen, 2002). Sound levels are recorded using the "A" scale in the sound-level meter and are expressed in terms of decibels (A) or dB(A) .The A scale is a filter that responds like a human ear. A 3-dB(A) increase in

sound level doubles the sound-pressure level. Therefore, for every 5-dB(A) increase in sound level, the permissible exposure time is cut in half. In other words, at 95 dB(A), the allowable exposure time is only four hours. It is not uncommon to have tractor sound-level reaching 95 dB(A).(Grisso et al., 2007) Tractor driver farmer had more often high frequency hearing loss when compared to non tractor driver farmer. The noise levels observed on tractors in different operations were in the range of 90–110 dB (A) (Kumar et al., 2005). Sound levels that cause hearing loss begin at about 85 dB(A). Hearing loss occurs more quickly with louder noise. OSHA Standards consider sound measured at 85 decibels or higher as damaging to the eardrum and therefore a risk to hearing (Anonymous, 2004). It has been investigated (Anonymous 2010) that 30 percent of Sweden's farmers suffer from hearing loss. Similar results to those from Sweden were found in a study conducted by University of Iowa in the United States, indicating that American farm workers are faced with the same noise problems in their daily work. An investigation by Dewangan et al (2005) for determination of SPL on 18.7 and 26.1 kW tractors and 4.6 and 6.7 kW hand tractors during field operations with various implements, revealed that both tractors produced the noise of 92 dB(A) in the working zone of operator. The SPL of the hand tractor was about 2 dB(A) higher than that of the tractor. The SPL during field operations at operator ear level increased with increase in engine speed and forward speed.

Celen and Arm (2003)found that the maximum SLP of 97.1 dB(A) was in exhaust pipe and the minimum of 79.7 dB(A) at the bystander ear. An increase of 3 dB(A) was measured for engine speed changes from 1000 to 2000 rpm. Durgut and Celen (2004) measured an 96.6 dB(A) at the drivers ear but a minimum of 67.7 dB(A) for the surrounding. They also found a 6 dB(A) difference when engine speed changed from 1000 to 2000 rpm. According to Aybek et al (2010), statistical analyses showed that type of operation, type of cabins, and operation x cabin interactions were statistically significant at ($P < 0.01$). The use of original cabins had a greater effect in decreasing average sound pressures and resulted in more efficient noise insulation, especially at higher center frequencies compared to field installed cabins. Sound pressure levels at 4000 Hz center frequency was reduced 2–13 dB and 4–18 dB by using a field-installed cabin and an original cabin, respectively. It was concluded that depending on the cabin types used, the operators could usually work from 4 to 6 h a day without suffering from noise induced inconveniences while 2–3 h is permissible for plowing and forage harvesting on tractors without cabins. In reference Anonymous (2009) it is stated that No Member State may refuse to grant EC (European Council) type-approval or national type-approval of any type of tractor on grounds relating to the driver-perceived noise level if that level is within the following limits: 90 dB(A) in accordance with Annex I, or 86 dB(A) in accordance with Annex II. Individual tasks which exceeded 85 dB(A) TWA-8 (NIOSH) identified by researchers were Tilling/plowing, Planting, other farm activities (Milz, 2006)

Equation (1) is given for safe exposure time to noise,

$$t = \frac{8}{2^{\frac{SPL-85}{3}}} \quad (1)$$

(Anonymous, 1996) where t= hours of exposure per day. An experiment in Croatia by Goglia and et al (2005) showed that by ISO 4872, 6393 and 362 standards, the noise level did not exceed the limit values. However, the noise level at the operator's position at full load and at nominal load exceeded the limits. Noise levels of 155 tractors on 36 farms were studied (Holt et al., 2006) The range of noise levels at the driver's ear with radios off and windows closed (if so equipped) was from 78 to 103 dB(A). Seventy-five percent of tractors without cabs had noise levels in excess of 90 dB, compared to only 18% of tractors with cabs. The use of a radio adds an average of 3.1 dB of noise. A specially selected group of 45 farm tractor drivers were examined in order to estimate the degree of occupational hearing loss (Holt et al., 2006). The drivers, aged 21-50 years, were employed on multi production farms. The study showed that the operators under study had statistically worse hearing within the range of high frequencies (3-6 kHz), especially those aged over 30 years. A study was carried out when a fabricated cab was added to an agricultural tractor (Abd-el-Tawwab et al., 2000). The fabricated cab was selected after a comprehensive series of experimental tests carried out on a variety of cab constructions. The results were discussed from the view point of obtaining the influence of the tractor driving parameters (road speed, gear-shift, engine speed and tractive effort) on the noise measured inside the tractor cab and over the frequency range up to 2000 Hz. In April, however, the occurrence of high total exposure values was due to intensive field activities (plowing, harrowing, sowing), and prolonged exposure to this factor (Aybek et al., 2010). In the seasons of the year

analyzed, high equivalent exposure values were observed within the range: 5.53-6.61 Pa^2 h (some polish standards). Mean value for this parameter for the whole year reached the value of 4.27 Pa^2 h (standard exceeded 4.3 times). This value is equivalent to a mean exposure level equal to 91.3 dB.

2. Material and Methods

Two types of tractor a 2-wheel drive MF399 with 62 kW PTO power without cabin and a Valtra T170, 184 kW power with cabin were tested. No load and loaded with mouldboard plough were tested for noise level at different forward speed. The measurements were taken at the driver ear and at a distance 7.5m from centre axis of tractor according to OECD standards(Anonymous 2010). A sound meter of type Lutron SL4013 equipped with capacitance microphone was used. The test course was a plot of 100 m long by 40m wide in open field. Each experiment data was recorded with 9 replicates. Data was analyzed based on factorial experiment with MSTAT-C software.

3. Results

The results are shown in Graphs 1-6 and tables 1-2. Comparison results were as follows:

1. Comparison between noise level in different forward speeds for each tractor with no cabin and open window cabin on driver's ear and bystander for no load and loaded cases

1.1. At driver's ear.

1.1.1. The noise level at 2.9 km/h for no load open cabin (graph 2), loaded no cabin (graph 3) and open cabin (graph 4) was significantly greatest among the related speed ranges as shown in Graph 1 and in third row in table 1. It looks like the 2.9 km/h speed is a threshold after which the SPL (Sound Pressure Level) drops although exception is seen in graph 3. Also although the trend is true for graph 1 but the SPL rises for 6.1 km/h speed. The permissible exposure time were 1.9, 2.5, 0.9 and 1.9 h/day for graphs 1, 2, 3 and 4 respectively as is shown in Graph 5 and in last row in table 1.

1.1.2. No load no cabin case. With a mean value of 87.72 dB(A) no significant difference was observed at 1% level for speeds up to 3.7 km/h; but the SPL for 6.1 km/h was significantly higher. The permissible exposure time was 4 h/day and 1.9 h/day respectively.

1.1.3. No load open cabin case. No significant difference in SPL at speeds 1.9, 2.2 and 6.1 km/h with a mean value 83.1 dB(A). However, significant difference was observed between 2.9 km/h and 3.7 km/h speed. The calculated exposure time for the latter speed was 6 h/day.

1.1.4. Loaded no cabin. No significant difference between SPL of 1.9, 2.9 and 3.7 km/h with mean value 93.5 dB(A) and 1.1 h/day allowed exposure time. Also no significant difference between 2.2 and 6.1 km/h but; a permissible exposure time about 6.3 h/day.

1.1.5. Loaded open cabin. No significant difference between SPL for 3.7 and 6.1 km/h with mean 89.2 dB(A) and permissible exposure time of 5.4 h/day. Also no significant difference between the SPL for 1.9 and 2.2 km/h with mean 83.7 dB(A). Bar graph for all exposure times are shown in Graph 5.

1.2. At bystander's ear.

Results for this case are shown in Graph 2 and columns 4,5,7 and 8 of table1. The graphs 1-4 in Graph 2 shows ups and downs but all SPL are lower than the safe level, 85 dB(A) which is set by NIOSH standards. Therefore, there is no limitation on the bystander's exposure time. For open cabin case, no load and loaded (graphs 2 and 4), the sound level increases up to speed 2.9 km/h but decreases after ward which is the same pattern for the same case at the driver's ear. For no cabin, no load (graph 1), the SPL is almost increasing but up to 3.7 km/h speed which does not resemble with the one at the driver's ear. However, the graph 3 follows the same pattern although with a wide gap with respect to the no load case. Graph 4 shows the bar graph for these cases. Allowable exposure time is shown in Graph 6.

2. Comparison between no cabin and open cabin in each forward speed for no load and loaded at driver's ear as well as at bystander. With reference to table 2:

2.1. At 1.9 km/h forward speed.

2.1.1. There was significant difference between the no cabin and open cabin noise level at the driver's ear (Table 2). Considering the doubling noise for a difference of 3 dB(A) [12], the sound level increases more than double at the driver's ear for no cabin loaded tractor compared to open cabin tractor (fourth column in table2). SPL for all cases was lower for open cabin compared to no cabin.

2.1.2. Significant difference was observed for loaded tractors at the bystander ear but; not so for the no load case.

2.2. At 2.2 km/h forward speed.

Significant difference observed between the SPL for no cabin and open cabin at the driver's ear for no load and loaded tractors. Data for open window were lower than that for no cabin. The SPL at the bystander's ear were not significant in either cases.

2.3. At 2.9 km/h forward speed.

SPL differences were significant for only the no load case at the bystander ear.

2.4. At 3.7 km/h forward speed.

Significant difference for the no load case at the bystander ear and for loaded tractors at the driver ear.

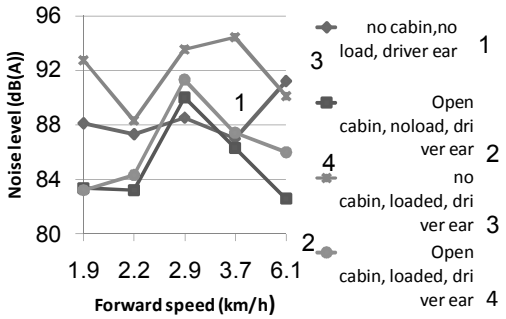
2.5. At 6.1 km/h forward speed.

No significant difference for only the loaded case at the bystander ear.

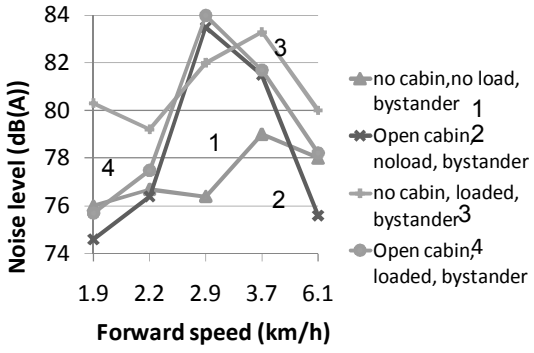
4. Discussion

For no cabin and open cabin both, data showed an increasing SPL with increase in forward speed up to 2.9 km/h as it was observed by Dewangan et al (2005) but; it decreased afterwards. One exception was for the no cabin loaded tractor at the driver's ear that the SPL continued rising up to 3.7 km/h and then dropped. The tractors were up shifted from Low 3 to Hi 1 to increase the speed from 3.7 km/h to 6.1 km/h. This shifting could have caused the drop after 3.7 km/h because of decreased torque and relieving the engine. No reason can be thought of for justifying the SPL drop after the 2.7 km/h. More research on this matter is recommended. Another exception was observed for no cabin, no load case at the driver's ear in which the sound level increased after the 3.7 km/h to 6.1 km/h. This may be attributed to inaccuracy in data because it contradicts the results for the other three cases. For the usual ploughing speed of 4.5 km/h to 5, in Iran, the driver, as calculated from equation (1) for 92 dB(A), should not be driving for more than 1.5 hours a day unless putting on some kind of ear protections. The result somehow agrees with Aybek and et al (2010) who allow ploughing for 2-3 hours a day. The maximum data point did not get over 96 dB(A) while Durgut and Celen (2004) reported it as 96.6. However, the noise level was in fact over 85 dB(A) for field operation as said by Solecki (2010). Holt et al (2006) obtained a value of 78 to 103 dB at the driver ear for tractors with closed cabin and with radio off while in this research, with even open cabin, the SPL did not reach 91 dB(A). Celen and Arm (2003) reported a minimum of 79.7 dB(A) noise level at bystander ear which compares well only for the no cabin, loaded case. The minimum here was less than 76 dB(A) for other cases with or without cabin. This might be due to the better make of engines with new technology.

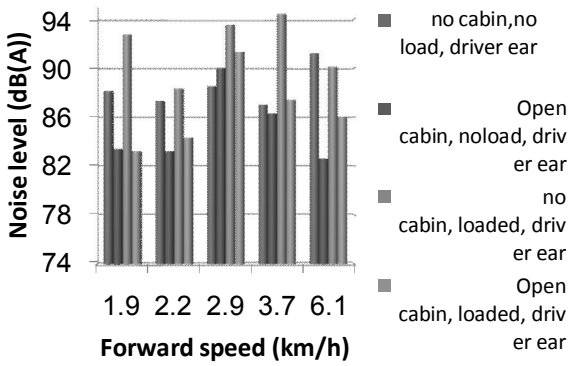
Finally, both the load and speed affects the sound level. While higher speeds over 4 km/h decreases the SPL, higher load on the engine increases it. For usual field operation, no harm at the bystander's ear but the driver at low speeds should either limit the exposure hours or wear some kind of ear protection while driving on no cabin tractor or even with cabin tractor if he is to frequently open the windows. Engines with new technology help in reducing noise.

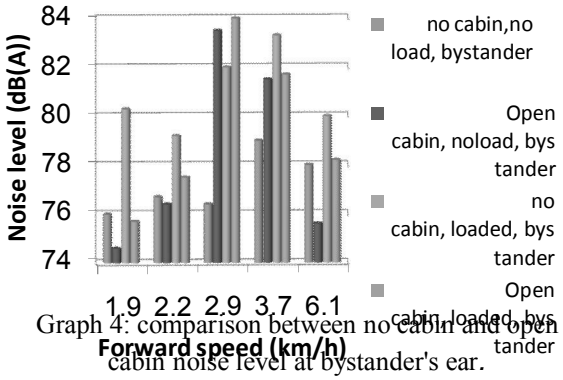


Graph 1: Mean noise level for different forward speeds, at driver ear.

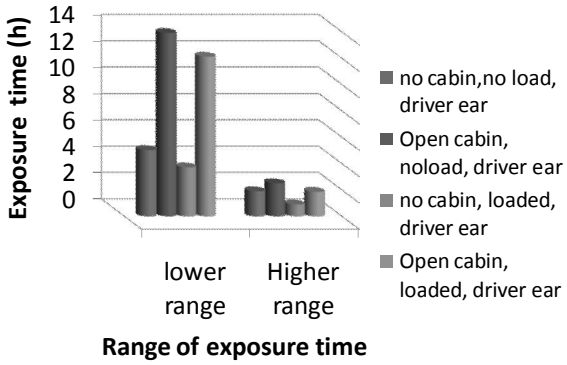


Graph 2: Mean noise level for different forward speeds, at bystander ear.

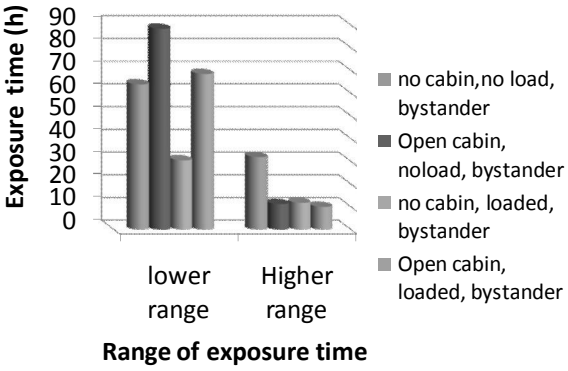




Graph 4: comparison between no cabin and open cabin noise level at bystander's ear.



Graph 5. Allowable exposure time for driver's ear.



Graph 6. Allowable exposure time for bystander's ear.

Table 1. Comparison of noise level in different forward speeds for each tractor with no cabin and open window cabin on driver's ear and bystander for no load and loaded cases.*

Forward speed km/h	Driver ear No load		Bystander ear No load		Driver ear loaded		Bystander ear loaded	
	No cabin	Open cabin	No cabin	Open cabin	No cabin	Open cabin	No cabin	Open cabin
1.9	88.1^{b}	83.4^{c}	76.0^{a}	74.6^{b}	92.7^{a}	83.2^{c}	80.3^{b}	75.7^{c}
2.2	87.3^{b}	83.2^{c}	76.7^{a}	76.4^{b}	88.3^{b}	84.3^{c}	79.2^{b}	77.5^{c}
2.9	88.5^{b}	90.0^{a}	76.4^{a}	83.5^{a}	93.5^{a}	91.3^{a}	82.0^{a}	84.0^{a}
3.7	87.0^{b}	86.3^{b}	79.0^{a}	81.5^{a}	94.4^{a}	87.4^{b}	83.3^{a}	81.7^{b}
6.1	91.2^{a}	82.6^{c}	78.0^{a}	75.6^{b}	90.1^{b}	86.0^{b}	80.0^{b}	78.2^{c}
Min.	87.00	82.60	76.00	74.60	88.30	83.20	79.20	75.70
Max.	91.20	90.00	79.00	83.50	94.40	91.30	83.30	84.00
Mean	88.42	85.1	77.22	78.32	91.8	86.44	80.96	79.42
SD	1.67	3.09	1.25	3.93	2.53	3.15	1.66	3.36
Lower exposure(h)	5.04	13.93	64.00	88.44	3.73	12.13	30.55	68.59
Higher exposure(h)	1.91	2.52	32.00	11.31	0.91	1.87	11.85	10.08

* Means with different letters at different forward speeds in each column are significantly different at 1% probability level.

Table 2. Comparison between no cabin and open cabin in each forward speed for no load and loaded at driver's ear as well as at bystander.*

Forward speed km/h	Driver ear No load		Bystander ear No load		Driver ear loaded		Bystander ear loaded	
	No cabin	Open cabin	No cabin	Open cabin	No cabin	Open cabin	No cabin	Open cabin
1.9	88.1^{a}	83.4^{b}	76.0^{a}	74.6^{a}	92.7^{a}	83.2^{b}	80.3^{a}	75.7^{b}
2.2	87.3^{a}	83.2^{b}	76.7^{a}	76.4^{a}	88.3^{a}	84.3^{b}	79.2^{a}	77.5^{a}
2.9	88.5^{a}	90.0^{a}	76.4^{b}	83.5^{a}	93.5^{a}	91.3^{a}	82.0^{a}	84.0^{a}
3.7	87.0^{a}	86.3^{a}	79.0^{b}	81.5^{a}	94.4^{a}	87.4^{b}	83.3^{a}	81.7^{a}
6.1	91.2^{a}	82.6^{b}	78.0^{a}	75.6^{b}	90.1^{a}	86.0^{b}	80.0^{a}	78.2^{a}

* Means with different letters at different loading and driver ear as well as bystander in each forward speed are significantly different at 1% probability level.

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Investigation of Micro Facies and Renovation of Old Formation Environment in Central Iran

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Abstract: The formation of Central Iran includes sequences of Dolomite, Dolomite ironstone and ironstone to upper Sanpaleosen-Ethosen in Zagros Mountains. For identification of micro facies of this formation from 216 m of its thickness in Dodaj Cutting (West of Shiraz) 120 thin sections was investigated. Performed desert and laboratory studies resulted to recognition of 7 micro facies and since this deposition is formed of benthic foraminifers, therefore by using of these suitable indices as a valuable tool, we can determine old environments of these deposits. Lack of turbidity, damming and reify and other studies show that Jahrom is formed in an ideal carbonate ramp. Because of high variety and plentifulness of benthic foraminifers, also this carbonate ramp is known as "Carbonate ramp system with plentifulness of benthic foraminifers".
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Keywords: Micro Facies, Central Iran, Old Formation Environment, Renovation.

1. Introduction

Jahrom deposition is considered a section of Tertiary Zagross basin to upper San Paleosen? – upper Eosen. Cutting of model of this formation is placed on shallow water in north wing of Jahrom mountain in south of Jahrom (Fars Province) in thickness of 268m and for the first time has introduced by James and Wind (1965). Old biological and ecological zoning of back Paleosen benthic foraminifers to former Myossen in north and south east of Shiraz by Khosrow Tehrani , et al (2007) is from the latest works has performed in relation with this deposition. Stratigraphy cutting under study is located in 30km of west of Shiraz and near Ahmadi village and through main road of Shiraz-Kharameh is in convenience and geographical coordinates E: 53°, 03', 40" and N: 30°, 30', 37". In this stratigraphy cutting, Jahrom deposition gradually is located on colored marens of Sachon Depost and gradually is covered by red color marens of hope (razak) deposition. For identification of micro facies of this formation in under study area, desert sampling was performed regularly and also with regard to change of facies. Finally, from samples 120 thin microscopic section provided which examined in laboratory. Nomination of carbonate stones in Dunham method (Dunham, 1962) was performed and classification of micro facies on the basis of Lassemi method (Lassemi, 1979) and Carozzi (Carozzi, 1989) was made. Finally micro facies with provided modes by Wilson (Wilson, 1986) and Flugel (Flugel, 2004) and other researchers was compared and 7 micro facies was recognized and finally also a model for old environment of this formation in case study area was suggested.

2. Discussion

In respect of fossilology, benthic foraminifers specially, large foraminifers such as Nummulites, Orbitolites, Somalina, etc. are found in this formation plentifully. Skelton parts of non-foraminifers such as soft bodies, Brachiopod, Beriosother, green algae, echinodermatous, coral and Estraked also there are together with foraminifers. Because of dulumiticity. More lower sections of Jahrom formation in under study area, plentifulness of foraminifers in this section relative to upper sections which is formed of limestone and dulumitic limestone is less. In the following it is referred to some of benthic foraminifers in under study area.

Alveolina pastisilata, Coskinolina sp., Lituonell sp., Miliolids, Nummunlties cf. subatacicus, Nummulites guettardi, Nummulites striatus, Ollssonina cribrosa, Orbitolites complanatus, Peneroplis sp. Lockhartia sp., Rapydionina urensis, Somalina stefaninii.

Generally, in respect to these foraminifers we can consider age of Eosen for Jahram formation in this cutting.

With regard to performed studies upon 120 thin cross, 7 types of micro facies with different ecologic combinations was identified. In bellow these micro facies in sequence from open sea to the shore have described and finally also a model for old environment of this formation in under study area in respect to these micro facies has been renovated.

MF7: Bioclastic nummulites wackstone

Main element in this micro facies, are nummulit benthic foraminifers about 2025 and bioclasts including briosother chips, green algae, bivalve shell and Estraked which are located as scattered in micrinic field. In some sections bioclasts are more than main element i.e. nummulit and name of micro facies is changed to Nummulites bloclast wackstone. Size and form of Nummulites specifically is in relation with type of sedimentary environment (Beavington-Penney et al., 2006) in a manner that stretched and large Nummulites are related to sedimentary sections (Beavington-Penney et al., 2006). So that stretched and large Nummulites are related to sections far from shore and deeper waters and is showing increasing the rate of space enable to sedimentation (Beavington-Penney & Racey, 2004). Existence of high percent of Hepaline foraminifers is indicating deeper sedimentary basin. But in samples which lack of foraminifers is index of Lagon environment in a general field explaining sedimentation bellow the rule of effect of wave (Rasser, 2005). On this basis we can result that this micro facies may has been formed under line of wave trace and in sections at the end of internal ramps and probably first sections of middle ramp. With regard to form of nummulites which are in lentil form and their small to medium size of them and also their thick walls we can conclude that they don't belong to very deep areas of the basin.

MF6: Rotaliid Wackstone

This facies mainly is including Rotalid foraminifers (Rotaliids and Lukartia) which forms about 15-20 percent of main volume of Allukems. Rotaliids are living in shallow waters (Geeel, 2000). Rotaliid species and Milluliids can re-product where the circulation of water doesn't replace them. With regard to mentioned cases, i.e. plentifullness of these hepaline foraminifers and decreasing of large benthic foraminifers with persulanoze crust and sedimentary mode represented by Beavington- Penny and Racey (figure 3), can indicates sedimentation of this micro facies in less shallow areas in relative to MF7.

MF5: Alveolina bioclast Nummulites wack/packstone

Main elements in this micro facies are including Nummulite about 15-20 percent and Alveolina with the percent about 10-12 and skeleton chips of non-foraminifers are about 12-15%. Alveolinas more are living in shallow basin (Beavington-Penny and Racey, 2004). In deed existent of Alveolina benthic foraminifers introduces internal section of ramp in carbonate platform (Papazzzoni et al., 2006). High percent of Nummulite together with Alveolina indicates that there has not been a barrier which prevents mixing of these large benthic foraminifers with Hepaline crust and persulanoze, but the rate of large foraminifers hepaline is more than persulanoze.

MF4: Bioclast Alveolina Nummulites Orbitolites wack/packstone

Main element in this Orbitolites micro facies is about 15-20% AND Nummulites And Alveolina is about 12-15% and skeleton chips of non-foraminifers is about 10-12%. In some samples sometimes name of facies is changed to Orbitolites Nummulites Alveolina wack/packstone which instead of Orbitolities, Alveolina is prevailing element. It is Wackstone texture that sometimes reaches to packstone. Accompanying Alveolina with Orbitolites is indicating a fairly disturbed and shallow environment (Reiss & Hottinger, 1984). Orbitolites also in back rife environments where in lagonic environments and near shore have the most expansion (Luterbacher, 1984) then existence of Orbitolites and Alveolina is indicating shallow of basin and as micro facies No. 3 accompanying them with Nummulites is indicating lack of a barrier which prevents of their mixing, but in these micro facies percent of foraminifers with porcelanoze crust is more which is indicating shallow of basin relative to micor facies No. 3.

MF3: Larger agglutinate forams Somalina wackstone

Main elements in this micro facies is including somalina foraminifer about 15% and large foraminifers with agglutinate crust (such as litveolena and gaskinovelina) is about 10-12%. Existence of Somalina which is part of large benthic foraminifers with persolanoze crust together with large foraminifers with agglutinate crust is indicating shallow of basin relative to previous micro facies.

MF2: Millolid bioclast wack stone

Main elements of this micro facies is including Millolids about 10% together with estraked, coral, green algae, Brakiopood and bivalve chips is about 10-12%. Milliolids mostly live in shallow waters and semi-sore to supra-sore with little disturbance, but in respect to bioclastic chips such as coral and green algae it is indicating shall and photic environments with special sore and temperature. Meanwhile considerable thickness of Estrakods crust in this micro facies can be a reason for more energy of basin. It means that probably this micor facies has been near the shore.

MF1: Dolostone

In this micro facies dullumite crystals in middle and fairly tiny sizes are seen as compressed and semi-compressed, deformed and dark to clear. Clearly we can see effect of initiate sedimentary texture and also we can see the effects of benthic foraminifers such as Millolids and chips of echinodermatous and anidritic evaporative cement in this micro facies which is indicating duplication of these dullumits. It sees that dullumits have been formed in tidy area.

3. Conclusion

After studying thin cross and identification of micro facieses and their comparison with Wilson and Fluggel standard facieses model and standard sedimentary models in case of Tertiary ramps it is considered that Jahrom formation in case study area is deposited in a carbonated ramp. Existence of benthic foraminifers with personaloze and hepalline crust with each other in these micro facieses indicates that during Jahrom formation deposition in case study area there has not been a barrier that can cause separation of these two environments. Therefore lack of this dam facieses and non-presence of turbidite facieses which is indicating slop ramps and indicates tininess of grains of most particles, specifies existent of a carbonate platform in type of ramp with mild slope (hemoclinal ramp) and ideal in this study. Also non-presence of facies related to deep areas which clear specification of that is existent of Plagic foraminifers and specifications of microfossils existent in that, shows that deepest part of this ram is related to shallow parts of open sea in internal ramp and probably is at the first of middle ramp. Therefore since Jahrom formation is full of benthic foraminifers, as a result by using of this valuable fossil content and other existent fossil part, in a limit we can renovate this formation. Of course in this case suggested models of carbonate ramps of researches such as Baxton & Pedley (1989) , Racey (1994) et al that by studying on foraminifers is performed, are used.

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Investigation of Effective Factors on Audit Committee Effectiveness from Perspective of Independent Auditors and Board Members

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Abstract: The mere presence of the audit committee does not necessarily translate into an effective monitoring body. As a result, the search for mechanisms to enhance corporate governance and increase the quality of financial reports has mostly focused on the structure of audit committees. The purpose of this research is an investigation of effective factors on audit committee effectiveness from perspective of independent auditors and board members. The statistical survey of two groups of independent auditors and board members of companies listed on the stock exchange. Data was collected using a questionnaire. 269 questionnaires from the independent auditors and board members of 347 questionnaires were analyzed. Results of this study showed that independence, activity, financial expertise, size, chart, support of board of director and experience caused the audit committee effectiveness.
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Keywords: Audit committee, Effectiveness, Independence auditor, Board of director

1. Introduction

During recent years, developed countries have been observed of appearance and evolution of audit committee.

This committee has been responsible for various needs of accounting and financial users, especially stockholders.

Expansion of international operations, intensification of stock companies activities for achieving competitive advantages, increase of liabilities resulted from damaging environment, the role and effect of management estimations in figures inserted into financial statement and its effect on it, lack of a base for investigation of management claims about efficiency of internal control structure by independent auditors, development of using computer systems followed by becoming more difficult monitoring this system controls have resulted in intensification of audit committee creation and application process.

The role of audit committee as an effective factor on increasing financial reports presentation desirability will be continued in the same manner as before and effective audit committee, will play key role in filling accrediting vacancy which today exist in financial reports.

Effective audit committees, cause increasing annual audited financial statements credit, committee effectiveness is in fact its ability to achieve its goals. (Zhang, 2006) in order that audit committee can operate effectively, must be independent of economic unit. To maintain independence, audit committee members should be elected from non-duty managers. They have such relation to economic unit that don't undermine their independence.

Furthermore, audit committee members must have sufficient and necessary financial and accounting expertise and knowledge. Without professional expertise and knowledge, members won't be able to understand economic unit financial reports and accounting information. Thus, can't operate effectively.

If audit committee including expert and independent members is inactive or low-active and inexperienced, certainly won't be effective. Moreover board of directors' support for audit committee increases its effectiveness. The purpose of this research is to investigate factors effective on audit committee effectiveness from viewpoint of independent auditors and board of board of directors' members.

2. Problem Expression

Nowadays with respect to growth and development of common stock companies, monitoring economic units operations and process has been more important than before. Providing reliable financial reports and accounting information is one of the essential needs of current evolving world. Audit committee is one of the mechanisms

which it's expected that can operate effectively in direction of keeping different groups' interests which use accounting information.

With respect to current evolving world, safeguarding investors' interests as capital suppliers and also the most important user groups of accounting information and financial reports have become more necessary than before. Nowadays, in some developed countries, they have established audit committee so that undertakes the role of monitoring procedures and the manner of economic units accounting and financial reports.

Created evolutions during recent years, in advanced countries have caused increasing auditors' independence of management when auditing. One of these changes is using audit committee in companies. This committee can operate as supervisory agent.

The member of this committee, are elected from non-duty managers and in the best situation, include managers out of company. Some duties of this committee include electing and deposing independent auditors, auditing services restrictions, auditors' findings investigation and resolving disagreements between management and auditors (Neal & Carcello, 2000). Aligning the interests of auditor and board of directors' non-duty members (auditing committee) is the best way to achieve an independent relationship between auditor and organization management.

Purposes of audit committee are:

- Assisting board of directors' members in order to perform their responsibilities.
- Providing better communication lines
- Improving auditors' internal and external independence
- Increasing credit and reliability of financial reports
- Improving and strengthening the role of non-duty managers.

In advanced countries, audit committee has become an important tool to control financial reporting procedure reliability.

Audit committee operates as a determinant factor in financial reporting process (Rainsbury et al. , 2009). Furthermore, the committee controls economic unit senior management and by preventing management from violating internal controls, such as preventing management deception, plays effective role. Thus, effective audit committees, cause increasing annual audited financial statements credit. Committee effectiveness is in fact its ability to achieve its goals (Yan Zhang et al, 2006). Moreover, the role of audit committee in risk management is also very important. Risk management is often done by audit committee through focus on goals, activities and work framework of organization (Livingston, 2005).

In this research we have tried to investigate factors which cause audit committee effectiveness. In other words, audit committee must have characteristics so that it can achieve short-term and long-term goals in best manner.

3. Necessity of Research

In order to make suitable decisions, financial reports should present information to assist investors, creditors and other users. But important matter is that in most cases, financial information suppliers and users of this information don't have the same goal and it's because of interest confliction between interested parties in companies.

With respect to performed researches, audit committee, as a supervising tool, plays important role in company. Since stockholders aren't able to monitor company continuously, so company board of directors takes this responsibility through entrusting audit committee with monitoring financial reporting process. Performed studies, have described the role of audit committee in improving internal controls and financial reporting very important.

Now, in order that audit committee can perform its supervising responsibilities in best manner, must be effective and it's the reason of performing this investigation that what properties should have audit committee so that can be effective.

4. History

Knapp (1987) conducted a study to identify the factors that could influence the likelihood that audit committees will support auditors in their disputes with management. The results of this study indicate that firms with an audit committee were more likely to support the auditor in auditor-management disputes compared with those with a non-Big 8 auditor.

Kalbers and Fogarty (1993) used the power theory to conduct a survey using audit committee members from 90 corporations to investigate the relationships between audit committee characteristics and ACE. The results of their analysis indicated that effectiveness included oversight of financial reporting, external auditors and internal control. Audit committee power within the organization came from a combination of written authority and the clear support of top management.

Cohen and Hanno (2000) found that external auditors made less favorable audit planning judgments in cases where the corporate governance structure included an audit committee that lacked technical experience and regular access to internal and external auditors without top management present.

Beasley et al. (2000) examined the association between the number of audit committee meetings and the likelihood of having fraud financial reports in the technology and health-care industries. The results of their study indicate that there was a negative relationship between the number of meetings and the likelihood of fraud. Their study indicated that while fraud companies generally held one meeting per year, non-fraud companies met two or three times each year. However, the number of audit committee meetings in both groups of companies is still less than the four meetings per year that was recommended by the Blue Robin Committee (BRC, 1999).

Song and Windram (2000) found that UK companies with an audit committee with a higher level of financial literacy are less likely to have financial reporting problems.

Abbott and Parker (2000) found that firms with independent audit committees were more likely to select industry-specialist external auditors. This may reflect the desire of such audit committees to reduce their members' reputational losses, which, in turn, will enhance audit quality; and indicated that firms with audit committees that met at least twice per year were less likely to be sanctioned by the SEC for financial reporting problems.

Vafeas (2001) examined audit committee composition in terms of independence and experience (expertise) and tried to identify the determinants of audit committee appointments. The results of this study indicate that while the likelihood of audit committee appointment increases with the degree of outside directors' independence, such likelihood decreases with compensation committee membership, other committee membership and the length of board tenure. However, audit committee appointments were not related to equity holdings and the number of other directorships; and highlighted the need to examine the influence of audit committee composition not only in terms of independence and expertise, but also in terms of financial literacy, on audit committee performance and the quality of the financial reports.

Archambeault and DeZoort (2001) examined the association between audit committee independence and suspicious auditor switches1. They found that companies with a suspicious audit change had a smaller percentage of independent directors on the audit committee compared to these with a non-suspicious auditor change; and found that companies with suspicious auditor switches had fewer experts on their audit committees compared to these with non-suspicious audit switches; and investigated the impact of audit committee size on suspicious auditor switching. They found a negative association between audit committee size and suspicious auditor switching; and concluded that companies with an audit committee that met more frequently were less likely to commit a suspicious auditor switch compared to companies with an audit committee that met less frequently.

Raghunandan et al. (2001) surveyed chief internal auditors and found that audit committees with at least one member possessing an accounting or finance background were more likely to have longer meetings with their chief internal auditor, to provide private access to him or her and to review internal audit proposals and results.

Abbott et al. (2002) found that companies with independent audit committees were less likely to be sanctioned by the SEC for fraudulent or misleading financial reporting.

Carcello et al. (2002) examined voluntary audit committee disclosures under the new disclosure requirements implemented in 2001 by the SEC and the securities exchanges such as NYSE. They found voluntary disclosure of audit committee activities to be more common for depository institutions, larger companies, NYSE listed companies, and companies with more independent audit committees.

Felo et al (2003) found a positive association between audit committee size and financial reporting quality. These studies provided support for the use of audit committee size as a proxy for the available audit committee resources.

Bedard et al. (2004) investigated the association between different audit committee characteristics and earnings management. They found that aggressive earnings management is negatively associated with the presence of an independent audit committee; and found that aggressive earnings management is negatively associated with the financial and governance expertise of audit committee members; and concluded that aggressive earnings management is negatively associated with the presence of a clear mandate defining the responsibilities of the audit committee

Chen et al. (2005) examined the relationship between independent audit committees and audit quality using the ASX Top 500 (at the time of testing the list contained 510 firms) in 2000. They found that the ASX Top 500 companies with high percentages of non-executive directors on their audit committees were more likely to hire a specialist (high quality) auditor compared to these with low percentages of non-executive directors on their audit committees

DeFond et al. (2005) investigated the market reaction to the appointment of directors with financial expertise to the audit committee. They found a positive market reaction to the appointment of directors with financial expertise to the audit committee.

The Treadway Commission's Report offered 11 specific recommendations designed to enhance the effectiveness of audit committees:

1. Audit committees should have adequate resources and authority to discharge their responsibilities.
2. Audit committees should be informed, vigilant, and effective overseers of the company's financial reporting process and its internal control system.
3. Audit committees should review management's evaluation of the independence of the company's public accountants.
4. Audit committees should oversee the quarterly as well as the annual reporting process.
5. The SEC should mandate the establishment of an audit committee composed solely of independent directors in all public companies.
6. The SEC should require committees to issue a report describing their responsibilities and activities during the year in the company's annual report to shareholders.
7. A written charter for the committee should be developed. The full board should approve, review, and revise it when necessary.
8. Before the beginning of each year, audit committees should review management's plan to engage the company's independent public accountant to perform management advisory services.
9. Management should inform audit committees of any second opinions sought on significant accounting issues.
10. Together with top management, the audit committee should ensure that the internal auditing involvement in the entire financial reporting process is appropriate and properly coordinated with the independent auditor.
11. Annually, audit committees should review the program that management establishes to monitor compliance with the company's code of ethics.

This report further recommended that all public companies be required by the SEC to establish audit committees composed solely of non-executive directors. However, the SEC did not respond to such recommendation (Solomon 1978).

The *Sarbanes-Oxley Act 2002* is the single most important piece of legislation affecting corporate governance, financial disclosure and the practice of public accounting since the US securities laws; in this Act, special attention was given to the audit committee as one of the most important corporate mechanisms to enhance the integrity of financial reports. In

Section 301, the following requirements are recommended:

• Each member of the audit committee shall be a member of the board of directors, and shall otherwise be independent.
• The audit committee shall be directly responsible for the appointment, compensation, and oversight of the work of the external auditor.
• The audit committee shall establish procedures for the "receipt, retention, and treatment of complaints" received by the firm regarding accounting, internal controls, and auditing.
• Each audit committee shall have the authority to engage independent counsel or other advisors, as it determines necessary to carry out its duties.
• Each firm shall provide appropriate funding to the audit committee.

5. Assumptions

In order to investigate research subject, there were propounded 7 theories each of which is in direction to research main goal assessment.

Theories are derived from theoretical structure, so have necessary credit in order to be tested. To test theories, before propounding theories, technical literature such as books, reliable and foreign scientific researches and publications concerning research subject were studied and through obtained information, subject related ideas were discussed as 7 theories:

1. There is meaningful relation between audit committee members' independence and audit committee effectiveness.
2. There is meaningful relation between audit committee members being active and audit committee effectiveness.
3. There is meaningful relation between existence of at least one accounting or financial management specialist and audit committee effectiveness.
4. There is meaningful relation between existence of audit committee charter and audit committee effectiveness.
5. There is meaningful relation between board of directors support for audit committee and audit committee effectiveness.
6. There is meaningful relation between audit committee members' experience and audit committee effectiveness.
7. There is meaningful relation between audit committee size and audit committee effectiveness.

6. Method of Research

The method used in this research is survey method. Date has been obtained through distributing a questionnaire which justifiability and durability have been confirmed. The questionnaire has been set based on 7-section Likret spectrum. Stages of this research are so that firstly variables were specified based on research literature. Then there were considered 2 statistical communities for research, the first community include board of directors' members

of accepted firms in Tehran Stock Exchange and second community includes independent auditors.

After extracting desired data through questionnaire distribution, research theories were analyzed by using of SPSS software.

7. Statistical Community and sample

First group statistical community includes board of directors' members and second group statistical community includes independent auditors. On the basis of the list released by Tehran Stock Exchange in 1390, 453 companies were accepted in Stock and with regard to 10 managers for each companies, it was considered that each statistical community in this research includes 4530 persons. Based on number of independent auditors which has been declared by audit organization, it was considered that second group statistical community includes 1750 persons.

Regarding research method and theories, Cochran formula was used to determine sample volume. Sample number for first group was obtained 355 and for second group was obtained 272.

8. Data Analysis

The main purpose of this research is to investigate effective factors on audit committee effectiveness between 2 groups of independent auditors and board of directors' members of accepted companies in Stock Exchange. In order to investigate research theories, one-sample T-test was applied. Theories were tested at 95% of confidence level. Furthermore, with regard to designed questionnaire format which was 7-item, average has been considered 4. Variance analysis test was used to determine the relation between two respondent groups and research theories.

For independent auditors:

H_0: $\mu_a <= 4$ Contradictory claim

H_1: $\mu_a > 4$ Claim

For board of directors' members:

H_0: $\mu_m <= 4$ Contradictory claim

H_1: $\mu_m > 4$ Claim

Variance analysis test is used to compare influence of a grouping independent variable over a dependent quantity variable. In this research, independent variables are board of directors' members and independent auditors and dependent variables are research theories. The purpose of performing this research is to determine is there any difference between independent auditors and board of directors' members' responses to questionnaire technical questions? **H_0: $\mu_a = \mu_m$ H_1: $\mu_a \neq \mu_m$**

Chart 1: Theories test results

Theory	Group	Average	T-Statistics	Df	Sig	Confirmed/Rejected
1	Auditors	5/9950	5/9950	268	0/000	Confirmed
	Board of directors	5/7474	5/7474	346	0/000	Confirmed
2	Auditors	5/9229	5/9229	268	0/000	Confirmed
	Board of directors	6/2186	6/2186	346	0/000	Confirmed
3	Auditors	6/2416	6/2416	268	0/000	Confirmed
	Board of directors	6/2891	6/2891	346	0/000	Confirmed
4	Auditors	5/8501	5/8501	268	0/000	Confirmed
	Board of directors	5/6590	5/6590	346	0/000	Confirmed
5	Auditors	5/9164	5/9164	268	0/000	Confirmed
	Board of directors	5/9063	5/9063	346	0/000	Confirmed
6	Auditors	5/8297	5/8297	268	0/000	Confirmed
	Board of directors	5/8622	5/8622	346	0/000	Confirmed
7	Auditors	5/2416	5/2416	268	0/000	Confirmed
	Board of directors	5/3516	5/3516	346	0/000	Confirmed

9. Research Findings

By performing this research and testing its theories, following results have been obtained.

In order to have effective audit committee, it's necessary:

- Audit committee members include company non-duty managers
- Board of directors doesn't be a member of audit committee
- To organize regular meetings of audit committee during the year
- Members regular participation in meetings
- To spend enough time to understand company and preparing oneself for meetings
- Timely distribution of agenda and background information
- To have impartial and reasonable questioning attitude towards issues
- To invite non-member (independent auditor, internal auditor and so on) to

meetings, without chairman attendance, if necessary.
- Key persons attendance (managing director, financial manager and so on) in meetings
- To record clearly decisions made in meetings
- Presence of at least one accounting or financial management specialist member
- Members must be able to read, comprehend and understand financial statements
- Training related to financial reports for people who have studied in fields other than accounting or financial management
- To have formal written authority
- Confirming audit committee chart by board of directors
- Reviewing audit committee chart annually and recommendation of necessary changes to board of directors
- Not to fear consequences of decisions made by audit committee
- Considering enough fees by board of directors for audit committee
- Considering organization power for audit committee
- Inviting audit committee to formal meetings, seminars and briefings of foreign auditors and company attorneys
- Having enough resources for utilization of legal consultants' services, if necessary
- Not to limit audit committee members election to one period
- Trainings related to economic unit regulations for audit committee
- Presence of experienced and aware persons in meetings

10. Recommendations for Future Researches

- Investigating the effect of audit committee existence on attracting foreign investors for investment in Iran economic units
- Investigating the effect of number and expertise of audit committee members on audit committee performance
- Determining possible and economical ways, for removing obstacles of audit committee establishment in Iran economic units
- Investigating the effect of audit committee on operational audit committee effectiveness and efficiency
- Investigating effects of audit committee existence on internal audit unit impartiality
- Investigating benefits of establishing effective audit committee in companies
- Investigating factors other than investigated ones in this research, which cause effectiveness of audit committee.

Chart 2: Variance Analysis Test Results

Theory	Situation	Sum of square	df	Mean square	F	Sig	Meaningful difference
1	Between groups	9/296	1	9/296	7/659	0/006	Has
	Intergroup	745/290	614	1/214			
	Total	745/589	615				
2	Between groups	13/258	1	13/258	28/860	0/000	Has
	Intergroup	282/074	614	0/459			
	Total	295/333	615				
3	Between groups	0/342	1	0/342	0/604	0/438	Doesn't have
	Intergroup	347/949	614	0/567			
	Total	348/291	615				
4	Between groups	5/533	1	5/533	6/483	0/011	Has
	Intergroup	524/045	614	0/853			
	Total	529/578	615				
5	Between groups	0/015	1	0/015	0/027	0/870	Doesn't have
	Intergroup	348/907	614	0/568			
	Total	348/923	615				
6	Between groups	0/160	1	0/160	0/278	0/598	Doesn't have
	Intergroup	353/218	614	0/575			
	Total	353/378	615				
7	Between groups	1/832	1	1/832	0/953	0/329	Doesn't have
	Intergroup	1180/400	614	1/922			
	Total	1182/232	615				

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SOCIAL CAPITAL DIMENSIONS AND FOOD SECURITY AMONG FARMING HOUSEHOLDS IN OGUN STATE, NIGERIA

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ABSTRACT: Despite an annual 2.5% growth in food production in Nigeria, food insecurity at the national and household level is dismal and on the increase from 18% in 1986 to 40% in 2005. With several studies on social capital and welfare in Nigeria, there is a dearth of information on the role that social capital plays in mitigating food insecurity. This study examined effects of social capital on food security among farming households in Odeda LGA of Ogun state, Nigeria. A multistage sampling technique was used to select 116 households in the study area in 2010. Data analysis was done using descriptive statistics and Probit regression. The food security line was ₦2,155.74 per month per adult equivalent. Based on this, 45% of the total sampled households were food secure while 55% were food insecure. Food secure households exceeded the calorie requirements by 19% while food insecure households fell short of calorie requirements by 28%. A unit increase in social capital ($p<0.01$) increases the probability of household to be food secure by 0.0991 while a unit increase in household size ($p<0.01$) and level of education of household head ($p<0.01$) decreases the probability of household to be food secure by 0.3482 and 0.1367 respectively. Disaggregation of social capital into its dimensions shows that cash contribution positively and significantly affects food security of farming households. Consistent with our a priori, households with higher levels of social capital are less likely to experience food insecurity.
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Keywords: Social Capital, Food Security, Farming Households, Ogun State.

INTRODUCTION

Achievement of food security for all remains a huge challenge for several developing countries like Nigeria. Food is a basic necessity of life. The importance of food at the household level cannot be overemphasized. Food accounts for a substantial part of a typical Nigerian household budget and it has been established that the quantity and quality of food consumed by households affect their health and economic well being (Adesimi and Ladipo, 1979). Hunger in sub-Saharan Africa is as persistent as it is widespread (FAO, 2006). Of the estimated 923 million undernourished people in the world, about 200 million of them in Sub-Saharan Africa (FAO, 2009) and in Nigeria, an estimated 8 percent of the 140million strong population was estimated to be undernourished in the 2004-2006 period (FAO, 2009). Among the development problems facing Nigeria, food insecurity ranks topmost. The level of food insecurity has steadily been on the increase since the 1980s and in spite of the Millennium Development Goal target to eradicate extreme poverty and hunger and halve the incidence of extreme hunger between 1990 and 2015 (FAO, 2006), less than 5 years to the target year, available statistics still cast doubt on whether this goal could be achieved by 2015.

Food security "exists when all people, at all times, have physical, economic, and social access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life" (FAO, 1996/2001). The basic minimum level of nutrient requirement has been determined by the Food and Agricultural Organization to be 2450 kcal of energy per capita intake of which if consumed otherwise results in a state of malnutrition (FAO, 2007). Faced with higher and fluctuating food prices leading to food insecurity and social unrest on the global scene, food security at both the national and household level is dismal and in Nigeria, the percentage of food insecure households was reported to be 18 percent in 1986 and 40 percent in 2005 (Sanusi *et al*., 2006). It then becomes imperative that Nigeria urgently takes action to cope with immediate needs for food and build a stronger food system that can respond to future challenges. At the household level, food security implies adequate access to food over time. Food access is a function of the physical, social, and policy environment which determine how effectively households are able to use their resources to meet their food security objectives, however, a number of factors such as income, educational level, and household sizes are known to affect household food security as they directly affect economic access and the sustenance of such access.

In Nigeria, the production of food has not increased at the rate that can meet up with the food demand of the increasing population (Ojo, 2003). While food production increases annually at the rate of 2.5 percent, food demand increases annually at a rate of more than 3.5 percent due to high rate of annual

population growth of 2.83 percent (NBS, 1996). The apparent disparity between the rate of food production and demand for food in Nigeria has led to a food demand-supply gap, leading to a widening gap between the food available and the total food requirement and hence posing a threat to national food security. The problem of food and nutrition security in Nigeria has not been adequately and critically analyzed, despite various approaches at addressing the challenge. The enormous amount of fund spent in attempting to assure the food security of Nigerians without success calls for a fundamental review of the past approaches and achievements to see what lessons can be learned to re-strategize and develop an approach that will ensure that better progress is made towards achieving the first Millennium Development Goal. An examination of social capital may offer insights into ways to decrease the prevalence of food insecurity and hunger in Nigeria, especially amongst rural settings.

Social capital has been found to have major impact on the income and welfare of the poor by improving the outcome of activities that affect them. It improves the efficiency of rural development programs by increasing agricultural productivity, facilitation, the management of common resources making rural trading more profitable, and improving access of households to water, sanitation, credit and education in rural and urban areas (Narayan, 2002). Social capital refers to the institutions, relationships and norms that shape the quality and quantity of a society's social interactions. It is not just the sum of institutions which underpin a society; it is the glue that holds a society together. According to Coleman (1988), social capital can take on three forms: firstly, obligations and expectations which depend on the trustworthiness of the social environment, secondly, the capacity of information to flow through the social structure in order to provide a basis for action and thirdly, the presence of norms accompanied by effective sanctions. Socialcapitalresearch.com defines social capital as "the value of social networks, bonding similar people and bridging between diverse people, with norms of reciprocity." Thus social capital has three main dimensions: Bonding social capital referring to strong family ties, bridging social capital referring to weak ties among friends and acquaintances and more formal ties linking members of voluntary organizations. There is growing empirical evidence that social capital has the potential to mitigate food insecurity in many developing countries. In times of financial hardship, food shortages, unreliable rainfall or severe illnesses, various studies in Africa have shown that the social capital that people have access to make a big difference in their abilities to surmount these adverse events (Mtika, 2001; Muga & Onyango-Ouma, 2009). Social capital is built during interactions for social, economic, cultural and religious reasons and the main assumption is that networks built through these interactions have measurable benefits to participating individuals and will lead directly or indirectly to improved welfare (food security). Putnam (2000) argues that social capital has "forceful, even quantifiable effects on different aspects of our lives such as enhanced economic achievement through increased trust and lower transaction costs (Fukuyama, 1995) and improved child welfare (Cote and Healy, 2001). The cumulative result of the research indicates that the well connected are more likely to be "housed, healthy, fed, hired and happy" (Woolcock, 2001). Also, growing opportunity requires an expanding stock of capital. Following from this, is the need to complement acquisition of natural, physical and human capital with social capital. Social capital has the power to mitigate shocks to income and food supplies in times of crises. Generally, the severity of the shock to income and food supplies and what coping strategies families may choose to utilize to cope with the shock may depend primarily on the strength of the social networks they have access to.

In current literature however, there has been very little emphasis on the role that social networks play in mitigating food insecurity in Nigeria. Furthermore, there is little empirical information on the relationship between various dimensions of social capital and food security status of households. Consequently, this study therefore empirically determined the effects of social capital on food security status at the household level using farming households in Odeda LGA in Abeokuta, Ogun State-Nigeria.

Literature Review

The basic idea of "social capital" is that one's family, friends, and associates constitute an important asset that can be called upon in a crisis, enjoyed for its own sake, and/or leveraged for material gain. Social capital is an important collective resource people draw on in pursuit of well-being. Conversely, the absence of social ties can have an equally important impact. The level of participation and involvement within a group signifies the investment being made by individuals, an investment into themselves and their community. By contributing to a group, the social capital of households as a whole can appreciate while individuals continue to build trust, develop relationships and networks with other members and this may contribute to a higher quality and level of life satisfaction (Bryant and Norris, 2002). Kawachi *et al.* (1999) argues that social capital can increase the likelihood of access to various forms of social support during times of need. At the household level, households that know and trust their

neighbors may be more likely to borrow food, or reciprocate with childcare responsibilities. These seemingly trivial favours could conceivably make a large difference in terms of access to food, especially for low-income households. Households may have similarly limited financial or food resources, but households with higher levels of social capital are less likely to experience hunger. Food security at the national and global level tends to focus on the supply side of the food equation. The question often raised is: is there enough food (dietary energy) available? But availability does not assure access, and enough calories do not assure a healthy and nutritional diet hence, distribution of the available food is critical and if food security is to be a measure of household or individual welfare, it has to address access.

Thus scholars identified three kinds of social capital: bonding, bridging, and linking. Bonding social capital refers to relationships among members of a group or network who see themselves as relatively equal, for example, immediate family, close friends and neighbours or schoolmates. Bridging social capital refers to relationships among people and groups of people who are fundamentally different in age, socio-economic status, race/ethnicity, or education. Linking social capital represents the extent to which individuals build relationships with the institutions and people who have relative power over them (e.g. to provide access to services or jobs) thus enabling them to leverage a far wider range of resources than was previously available to them (Woolclock, 2001).

In this study, the Social Capital is therefore viewed as membership in local level institutions, regular meeting attendance, volunteering, entertaining, or active participation in group activities as well as through methods of mutual assistance that are inherent in a given society, representing a sustainable and long-lasting system, woven into the social fabric of the people. People's entitlement to food is assured during various crises through this mechanism.

METHODOLOGY

The study was carried out in Odeda LGA, Ogun State and data for the study were collected in 2010. Respondents were selected using multistage sampling technique. Odeda LGA was purposively selected and six villages (two each from each of the three sub zones of the study area -Odeda, Ilugun and Opeji) were chosen from which 116 respondents were selected randomly.

Descriptive Statistics

Descriptive tools such as frequency counts, mean and standard deviations, percentages were used to analyze food security status by socio-economic variables of respondents. In addition, food security and surplus indices were constructed.

Food Security Index

Based on the food security line and recommended daily calorie requirement, the food security index was computed using the Cost of Calorie function (proposed by Greer and Thorbecke, 1986). This method was used because of its simplicity. Households whose daily per capita calorie intake is up to 2450 kcal were regarded as food secure while those below 2450 kcal were regarded as food insecure households (FAO, 2007).

Ln X = a+bC …….... (i)

Where

X = Food Expenditure (₦)

C = Calorie Consumption (kcal)

$Z = e^{(a+bL)}$ …….... (ii)

Where

Z = Cost of minimum recommended energy level (₦) – Food security line for the study area

L = Recommended daily energy level (2450 kcal)

a = Intercept

b = Coefficient of Calorie Consumption

e = A mathematical constant (2.71828)

A household whose average cost of daily calorie consumption is equal to or more than Z is said to be food secure while any household with average cost of daily calorie consumption is lower than Z is said to be food insecure.

Surplus/Shortfall Index

The Index is given as:

$$P = {}^{1}/_{N} \sum_{j=1}^{m} G_j \quad \text{……..…….(iii)}$$

$G_j = (X_j - L)/L$ ……..……(iv)

Where

P = Surplus/Shortfall Index;

L = Recommended daily per capita requirements (2450Kcal.);

G_j = Calorie deficiency faced by household $_j$;

X_j = Per capita food consumption available to household $_j$;

N = Number of households that are food secure (for Surplus index) or food insecure (for Shortfall index).

This index measured the extent to which households were food secure or insecure.

Social Capital Variables

The aggregate social capital index was obtained via a multiplicative index of the three social capital dimensions (density of association, heterogeneity and participation in decision making)

and normalized to a maximum value of 100 (Grootaert, 1999).

Density of Membership: is captured by summing up the membership of associations by individuals in the household.
Meeting Attendance Index: is obtained by summing up the attendance of household members at meetings and relating it to the number of scheduled meetings by the associations they belong to. This value is then multiplied by 100.
Cash Contribution: is obtained by adding up the total cash contributed to the various associations the household belong to.
Labor Contribution: is the number of days that household members belonging to associations claimed to have worked for their associations.
Decision Making Index: is obtained by summation of the subjective responses of households on their rating in the participation in the decision making of the three most important institutions to them. The response is averaged across the three groups and multiplied by 100 for the household.
Heterogeneity Index: is an aggregation of responses of each household to questions on the diversity of members of the three most important institutions to the household. Questions are answered on whether members live in the same neighborhood, are same kin group, same occupation, same religion, same gender, same age group and same occupation. For each of the factors, a yes response was coded 0 and a no response was coded 1 and a maximum score of 11 for each association represents the highest level of heterogeneity.

Determinants of Food Security

Probit model constrains the estimated probabilities to be between 0 and 1 and relaxes the constraint that the effect of the independent variable is constant across different predicted values of the dependent variable. This is normally experienced with the Linear Probability Model (LPM) (Sebopetji and Belete, 2009). The probit model assumes that while we only observe the values of 0 and 1 for the variable Y, there is a latent, unobserved continuous variable Y* that determines the value of Y. The other advantages of the probit model include believable error term distribution as well as realistic probabilities (Nagler, 1994). We assume that Y* can be specified as follows:

$$Y^* = X'\beta + \varepsilon,$$

where $\varepsilon \sim N(0, 1)$. Then Y can be viewed as an indicator for whether this latent variable is positive:

$$Y = 1_{\{Y^* > 0\}} = \begin{cases} 1 \ if \ Y^* > 0 \ i.e. -\varepsilon < X'\beta, \\ 0 \quad otherwise. \end{cases}$$

Where
Y =Vector of dependent variable (1 for food secure households; 0 for food insecure households);
X =Vector of explanatory variables;
β =Probit coefficients;
μ_i =Random error
Probit regression model was used to estimate the food security status of households as a function of some independent variables/determinants.

The determinants/explanatory variables included in the model are:
X_1 = Household size (number);
X_2 = Age of household head (years)
X_3 = Gender
X_4 = Education Level
X_5 = Years of Farming Experience
X_6 = Income (₦)
X_7 = Marital Status
X_8 = Social Capital Index

RESULTS AND DISCUSSION

Household Characteristics and Social Capital Dimensions

As presented in Table 1, 94.8 percent of respondents are below 60 years of age hence in their economic active age. Attendance at meetings shows that on the average, households attend two out of every four meetings but attendance rate is highest for respondents between 51 and 60 years of age who have a meeting attendance index of 83.27%. It was observed that membership diversity, meeting attendance and cash contribution increased with years of formal education. The highest representation of cash contribution to various associations is within the age group of 51 and 60 years. Male household heads in the study area attend more association meetings than their female counter part; however, female heads had a higher average annual cash contribution of (₦19964.71) than males who contribute ₦16038.38 on the average yearly.

Table1: **Description of Social Capital across Socio-economic variables of the Respondents**

Variables (Household Head)	Frequency	Membership Density Index	Heterogeneity Index (%)	Meeting Attendance Index (%)	Cash Contribution (₦)	Labor Contribution (manday)	Decision Making Index (%)
Age (years)							
≤ 30	18 (15.5)*	2.78 (1.66)	46.13 (21.94)	72.37 (35.595)	13744.44 (17458.77)	5.44 (17.96)	35.19 (33.28)
31-40	39 (33.6)*	3.13 (1.61)	41.88 (20.76)	74.70 (21.87)	16312.82 (25903.17)	3.69 (6.79)	37.61 (28.54)
41-50	28 (24.1)*	4.79 (2.66)	54.65 (19.89)	83.27 (20.19)	24607.14 27846.10	5.86 (10.98)	51.19 (29.72)
51-60	25 (21.6)*	5.20 (5.50)	54.06 (31.56)	57.89 (38.90)	10432.00 (13756.41)	0.32 (0.95)	23.33 (31.55)
>60	6 (5.2)*	1.00 (0.00)	25.25 (1.56)	71.35 (39.94)	15633.33 (12500.51)	0.00 (0.00)	25.00 (13.94)
Gender							
Male	17 (14.7)*	3.00 (1.73)	44.56 (20.69)	56.20 (39.61)	19964.71 (17796.94)	3.53 (9.96)	37.25 (23.96)
Female	99 (85.3)*	3.95 (3.44)	47.87 (24.52)	75.43 (27.35)	16038.38 (23592.69)	3.58 (9.84)	36.70 (32.03)
Household size							
1-4	16(13.8)*	2.75(1.69)	36.17 (23.48)	64.53 (33.62)	13337.50 (13312.99)	4.38 (7.27)	25.00 (19.25)
4-8	70(60.3)*	3.44(2.22)	47.88(23.17)	75.05 (27.77)	19925.71 (26500.55)	4.43 (11.53)	40.24 (30.49)
9-12	27 (23.3)*	5.00 (5.26)	52.53 (26.15)	72.97 (31.69)	6777.78 (9146.22)	1.11 (5.77)	33.95 (37.41)
13-16	3 (2.6)*	7.33 (2.89)	49.49 (4.63)	55.56 (50.92)	45333.33 (16165.81)	1.33 (1.15)	44.44 (9.62)
Educational level							
No formal	41 (35.3)*	3.51 (2.65)	45.09 (27.91)	60.51 (39.60)	12360.98 (16871.23)	2.54 (6.93)	34.96 (32.02)
Primary	54 (46.6)*	3.80 (3.74)	46.02 (22.32)	76.54 (22 .35)	11096.30 (12585.75)	4.44 (11.81)	32.72 (30.02)
Secondary	19 (16.4)*	4.42 (3.24)	54.07 (18.71)	85.82 (14.63)	30463.16 (25983.42)	3.68 (9.55)	49.12 (29.12)
Tertiary	2 (1.7)*	4.50 (0.71)	68.18 (2.14)	89.02 (7.45)	121200.00 (0.00)	0.00 (0.00)	66.67 (0.00)

Figures in parenthesis are percentages

Dimensions of Social Capital in Odeda

Table 2a presents the social capital dimensions of the sampled households. In terms of meeting attendance, results show that an average of 72.61 % attendance by respondents and households contribute on the average ₦16,613.79 yearly as cash contribution to their respective associations. Participation in decision making shows good level of activity with a 73.56 % participation index on the average. The heterogeneity level indicates low level (47.39 %) of diversity of membership of associations.

Table 2a: Household Activity in Associations

Social Capital Dimensions	**Minimum**	**Maximum**	**Mean**	**Standard Deviation**
Meeting Attendance	0.00	100.00	72.61	30.04
Heterogeneity Index	0.00	84.85	47.39	23.94
Participation in Decision Making	0.00	200.00	73.56	61.78
Cash Contribution Index	0.00	121,200.00	16,613.79	22811.64
Labor Contribution Index	0.00	76.00	3.57	9.81

Some dimensions of cognitive social capital (Table 2b) found among the respondents showed that most households (89.7 percent) could count on their neighbours for some form of help in times of emergencies while 96.6 percent had at least one close friend who could be relied on in cases of emergency.

Table 2b: Variables of Social Network, Social Support and Social Interaction

No. of close friends	Frequency	Percent
0	4	3.4
1-5	89	76.7
6-10	21	18.1
11-15	2	1.7
Total	116	100.0
Count on neighbors	**Frequency**	**Percent**
Yes	104	89.7
No	12	10.3
Total	116	100

Depth of Food Security among the Respondents

Based on the recommended daily energy levels of 2450Kilocalories (FAO, 2007), the food security line for farming households in the study area was estimated at ₦69.54 per day per person (₦2,155.74 per month per person). Annually, this is equivalent to ₦25,034.4 per year per person. Results (Table 3) showed that 45 percent of the sampled households were able to meet the recommended daily per capita calorie requirement of 2450Kilocalories hence were food secure. About 55 percent of the households were food insecure, subsisting on less than the recommended daily per capita calorie requirement of 2450Kilocalories. The Surplus Index (P) shows that the food secure households exceeded the calorie requirements by 19 percent, while the Shortfall Index shows that the food insecure households fell short of the recommended calorie intake by 28 percent.

Table 3: Food Security Indices

Variables	Value
Cost of Calorie equation	ln X = a+bC
Constant	4.239
Slope coefficient	1.2 x 10^{-6}
Recommended daily Energy levels	2450Kcal
Food Security line Z: Cost of minimum energy requirements per	
Adult Equivalent	₦ 69.54 per day
	₦ 2,155.74 per month
	₦ 25,034.4 per year
Head count ratio (H)	0.55 (for food insecure households)
	0.45 (for food secure households)
Percentage households:	
Food secure households	45%
Food insecure households	55%
Surplus Index	0.19
Shortfall Index	0.28

Probit Model: Social Capital Dimensions and Household Food security

Unlike the basic model 1 that excludes the social capital indices; the inclusion of six social capital indices in the model 2 increases the pseudo R^2 from 0.2675 to 0.3029 (Table 4). Primary exogenous variables such as education, income of household head, and household size were statistically significant. The pseudo R^2 slightly increased in the model with the inclusion additive social capital indices suggesting that social capital plays a significant role in explaining variations in household food security status. A relative good performance is also recorded with multiplicative social capital index (Model 3).

Marginal effects of Social Capital on Household Food Security (Model 3)

Household size

Household size was a significant determinant of food security of respondent households with a marginal value of 0.35. This means that a one percent increase in household size will reduce the probability of household to be food secure by 35 %. This result is expected because increase in the household size implies that more people are eating from the same resources, hence, the household members may have less food to go round when compared with a smaller household size. The result is in line with the findings of Olayemi (1998).

Level of Education of household head

According to studies by Agbola, (2004) and Babatunde *et al* (2007), level of education of the household heads has significant effect on the probability of households to be food secure. Findings revealed that the level of education of household heads was a significant and negative determinant of households' food security status. A unit increase in the level of education of household head will reduce the probability of household to be food secure by 0.15. This suggests that a household with a well educated household head may not necessarily be food secure.

Income

Results show that income of household heads is a significant and positive determinant of households' food security status. A unit increase in the level of income of household head will increase the probability of household to be food secure by 0.0000864. This result is in line with studies by Babatunde *et al* (2007) who found out that the higher the household head's income, the higher the probability that the household would be food secure. This was as expected because all things being equal, higher income increases households' economic access to food.

Social Capital

Findings also confirm that social capital is significant and positively associated with household food security status at 10 percent level with a marginal value of 0.0991 hence, a percentage increase in trust

level of respondents will increase the household's probability to be food secure by 9.9 %. Households with higher levels of social capital are less likely to experience hunger. This is in line with other studies (Yusuf 2008; Okunmadewa *et al* 2005; Kawachi *et al*. 1999, Rose 2000; Mtika, 2001; Muga & Onyango-Ouma, 2009) which state that social capital has positive influence on welfare and is an important factor in improving the quality of life of households.

Table 4: Probit Result of effects of Social Capital on Food Security

	Basic Model 1	With Additive Social Capital Variables Model 2	With Multiplicative Social Capital. Model 3	Marginal Effects for (Model 3)
Age of Household Head	-0.0574 (0.69)	-0.0817 (0.90)	-0.0453 (0.53)	-0.0735
Squared Age	0.0002 (0.28)	0.0004 (0.50)	0.0000 (0.11)	0.0001
Sex of Household Head	0.4629 (1.02)	0.5272 (1.06)	0.5257 (1.14)	0.9349
Education	-0.0925 (2.63)***	-0.1387 (2.90)***	-0.0854 (2.35)***	-0.146 4
Marital Status	0.4800 (0.60)	-0.0424 (0.04)	0.6597 (0.79)	1.2929
Household size	-0.2131 (3.67)***	-0.2147 (3.34)***	-0.2026 (3.41)***	-0.3482
Farming Experience	-0.0148 (1.00)	-0.0156 (1.01)	-0.0134 (0.90)	-0.0213
Income	0.0000 (2.79)***	0.0000 (2.81)***	0.0000 (2.84)***	0.0000
Social Capital Index	-	-	-0.0056 (0.75)	-0.0124
Heterogeneity Index	-	-0.0081 (0.93)	-	
Meeting Attendance	-	0.0005 (0.08)	-	
Cash Contribution	-	0.0000 (1.90)**	-	
Labor Contribution	-	0.0035 (0.19)	-	
Decision Making Index	-	-0.0020 (0.71)	-	
Membership Index	-	0.0433 (0.69)	-	
Number of Observation	116	116	116	
Pseudo R^2	0.2675	0.3029	0.2710	
Log likelihood	58.4420	55.6151	58.1608	
Constant	2.6368	4.1204	2.2643	

Figures in parenthesis are t values

*** significant at 1%, ** significant at 5%, * significant at 10%

CONCLUSION

The study shows that 45 percent of the sampled households in the study area were food secure while 55 percent of the households were food insecure. Hence, more farming households in the study area are food insecure than those that are food secure. Social capital is significant at 10 percent level and positively related to household food security status. Household food security decreases with increasing household size. The level of education of household heads was a significant and negative determinant of households' food security status, suggesting that a household with a well educated household head may not necessarily be food secure. The study concludes that social capital has positive influence on household food security and is an important factor in improving the quality of life of farming households.

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Explaining the Implications of Climate Change and the Associated Farmers' Risk Preferences in Nigerian Agricultural Economy

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Abstract: Climate change is a change in the statistical distribution of weather over periods of time that range from decades to millions of years. Agriculture however, is extremely vulnerable to climate change. Higher temperatures eventually reduce yields of desirable crops while encouraging weed and pest proliferation. Managing climate risk is especially important in agriculture not only for the direct impact that climate has on production, but also because most farmers tend to be risk averse. The fore knowledge that farmers' are mostly risk averse therefore calls for re-opening of an enabling policy environment that effectively considers the farmers perception of risk. This therefore provides a basis for making policy option towards adaptation to climate change given the farmers' characteristic socio-economic environment.

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Keywords: Implication; Climate; Farmer; Nigerian; Agricultural Economy

Introduction

Agricultural development and sustainability in Nigeria as could be obtained in other countries of the worlds is indeed a function of climate among other important factors. Invariably, climate has effects on what, when and where to produce as well as the level of output achieved. The multiplier effects of climate change are therefore recorded not only in the economic growth; food self sufficiency but the farmers' welfare who are the units from where decisions about food production activities are actually taken. Unfortunately, studies have severally shown that these farmers are characterized as poor and lack adequate resources for production. Hence, the farming households tend to obey a safety – first principle that assumes the individual's objective is to minimize the probability of experiencing a short fall in income below a certain initial level (Roy, 1952). For instance, overtime farmers have experienced severe losses or failure in their cropping activities. When these occur, the household's livelihood becomes threatened and subsequent investment in farming may be affected. Such household may reduce the scale of operation in the subsequent planting season, switch to another farming enterprise or embark on non- farm activities (Gordon and Craig, 2001).

Climate Change, Causes and Effects

Climate change is a change in the statistical distribution of weather over periods of time that range from decades to millions of years. It can be a change in the average weather or a change in the distribution of weather events around an average (for example, greater or fewer extreme weather events). Climate change may be limited to a specific region, or may occur across the whole Earth (Wikipedia, 2010). Climate change is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and which is in addition to natural climate variability observed over comparable time periods. This is in line with Odjugo (2007) that identified two factors as the causes of climate change. These are natural processes (biogeographical) and human activities (anthropogenic).

Climate change is one of the most important global environmental issues of our generation. It is distinct from natural climate variability in that it exists because of human activities that have altered the composition of the Earth's atmosphere. In his study, Odjugo (2007) described the trend in Temperature and Rainfall in Nigeria from year 1901 to 2005 (Fig 1 and 2). It was discovered that the temperature trend in Nigerian since 1901 shows increasing pattern (Fig 1). The increase was gradual until the late 1960s and this gave way to a sharp rise in air temperatures from the early 1970s, which continued till 2005. The mean air temperature in Nigeria between 1901 and 2005 was 26.6^{o}C while the mean temperature increase for the 105 years was 1.1^{o}C. This is obviously higher than the global mean temperature increase.

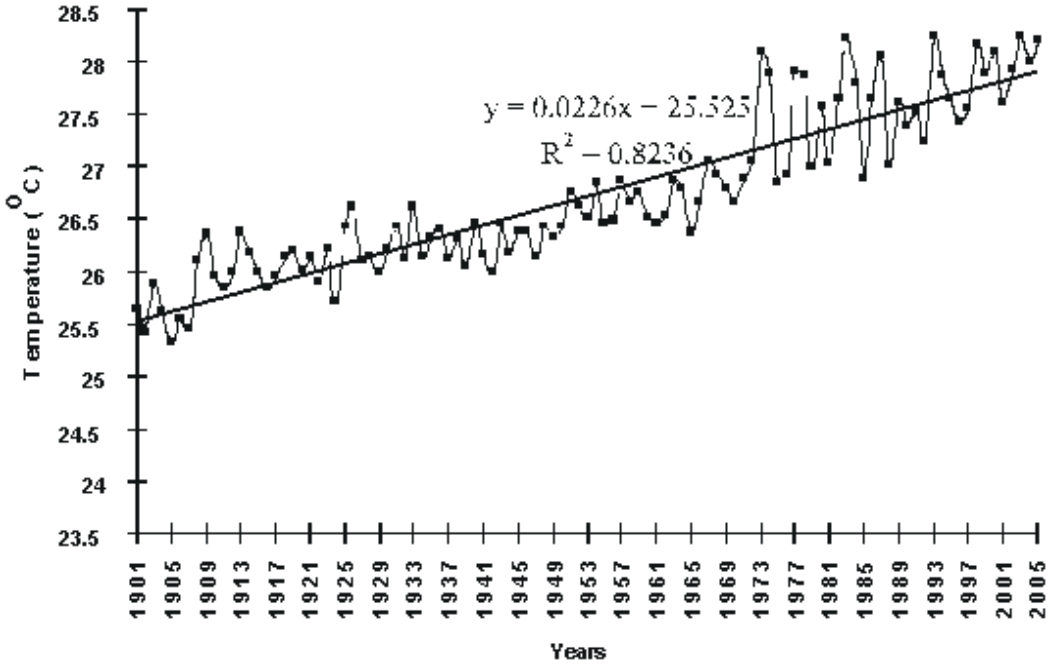

Fig. 1. Air temperature distribution in Nigeria between 1901 and 2005
Source: Odjugo (2010)

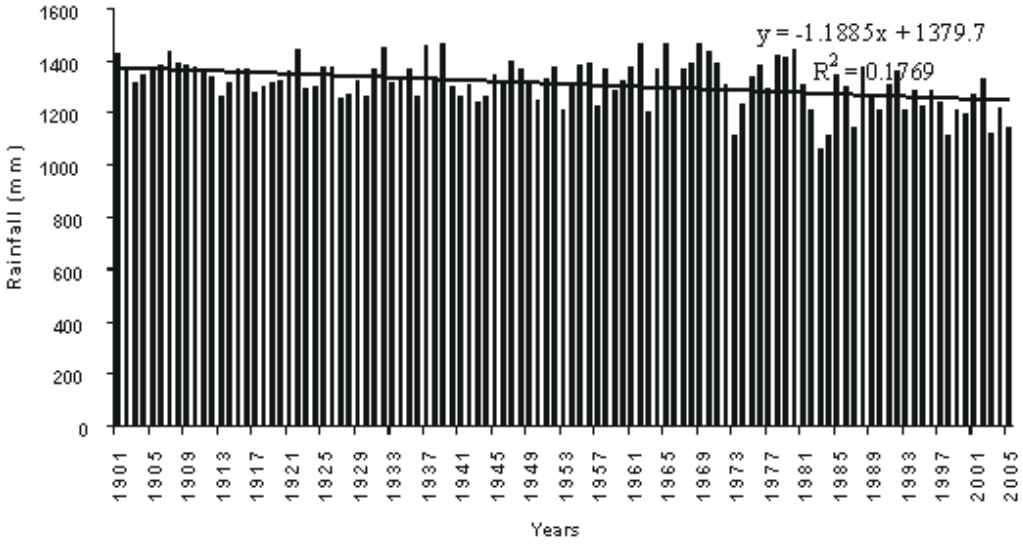

Fig. 2. Rainfall distribution in Nigeria between 1901 and 2005
Source: Odjugo (2010)

Climate change can lead to desertification, more intense storms, melting of the polar ice caps, and rising sea levels, changing the physical face of the Earth and the pattern of our everyday lives. While the possible consequences of climate change are alarming, there are many ways for every individual to take part in preventing these consequences from reaching their most dangerous potential (Economii, 2010).

Agriculture however is extremely vulnerable to climate change. Higher temperatures eventually reduce yields of desirable crops while encouraging weed and pest proliferation. Changes in precipitation patterns increase the likelihood of short-run crop failures and long-run production declines. Although there will be gains in some crops in some regions of the world, the overall impacts of climate change on agriculture are expected to be negative, threatening global food security. A number of empirical studies (Oyekale, *et al* 2007; Apata *et al* 2009; Ajetomobi and Biodun 2010 and Salimonu *et al* 2010) have been

carried out in recent times in Nigeria establishing the effects of climate change on agricultural production. The chorus is that of negative effect/impact.

Odjugo and Ikhuoria (2003) observed that Nigeria north of 12^{o}N is under severe threat of desert encroachment and sand dunes are now common features of desertification in states like Yobe, Borno, Sokoto, Jigawa and Katsina. The migrating sand dunes have buried large expanse of arable lands, thus reducing viable agricultural lands and crops' production. This has prompted massive emigration and resettlement of people to areas less threatened by desertification. Such emigration gives rise to social effects like loss of dignity and social values. Oyekale, *et al* (2007) studied the effects of climate change on cocoa production in Ondo state Nigeria. The study concluded that majority of the cocoa farms examined had declining yield with unfavourable climate changes. Low outputs from farms in south western Nigeria, as a result of low rainfall and increased temperature were also detected in the study of Apata *et al* (2009). Farmers in the study area were also abandoning mono-cropping for mixed farming in order to cope with losses in the earlier years. An in-depth analysis carried out by Ajetomobi and Biodun (2010) in Nigeria recently also revealed that Climate change has negative impacts on cowpea productivity in Nigeria over the years reviewed.

Risk Preferences among the Farmers

Risk is defined as a condition in which the results of any decision or course of an action are not definitely known but will probably fall within a known range (Montana and Charnov, 1987). It is also defined as the uncertainty of outcomes (Hardaker, 2000). It is a measure of the effect of uncertainty on the decision maker. It also refers to variabilities or outcome, which are measurable in an empirical or quantitative manner (Olayide and Heady, 1982).

The expected utility model has been used extensively to investigate behaviour under risk. The expected utility model views decision making under risk as a choice between alternatives. Decision makers are assumed to have a preference ordering defined over the probability distributions for which a number of axioms hold (Joost and Philip, 2001).

Attitudes towards risk can be divided into three types: risk averse, risk preferring and risk neutral (Henderson, 1980; Boehlje and Venon, 1984). Risk averters, or avoiders, are characterized as more cautious individuals with preferences for less risky sources of income or investment. In general, this individual will sacrifice some level of expected return in order to reduce the possibility of a loss. A person who is considered risk averse will likely have a low risk bearing ability. Their situation would be such that a large income loss would seriously disrupt or end the business.

Risk preferring individuals are characterized as more adventuresome with a preference for more risky business ventures. Risk preferers will select the alternative with some probability of a higher outcome. In order to get this higher income, the individual must also accept a probability of a lower outcome compared to the risk averter. The individual likely has a greater risk bearing ability and therefore is less concerned with the increased probability of a lower outcome and primarily focuses on the higher outcome potential. The risk neutral person is the limiting case between the risk averse and risk preferring individuals. He will select the alternative with the highest expected outcome, regardless of the probabilities associated with potential gains or losses. This person will have acceptable levels of risk bearing ability such that large losses are not of concern but at the same time, achieving the highest outcome is not the focus either. A number of studies (Adubi, 1992; Olarinde, 2004 and Salimonu, 2007) in Nigeria however submitted that majority of the farmers were highly risk averse.

Climate Change and Farmers Risk Preferences-The Nexus

Temperature and rainfall among others constitute major determinants physical nature and element of climate whose variation could have significant effects and impacts on agricultural productivity over time. The foregoing rather suggests climate change as a significant source of risk to the agricultural economy in particular. The IPCC (Intergovernmental Panel on Climate Change) in its 2001 assessment had grouped key vulnerabilities into five main 'reasons for concern' about climate change. These are: the risk to unique and threatened systems (ecosystems and human populations), the risk from extreme weather events, the (uneven) distribution of impacts, the global aggregate impacts, and finally the risks of large scale singularities (the 'big' or extraordinary events that would cause global-scale damage, such as loss of the Greenland ice sheet, or a shutdown of the Gulf stream).

Managing climate risk is especially important in agriculture not only for the direct impact that climate has on production, but also because most farmers tend to be risk averse. Risk aversion implies that farmers do not optimize their farm-plan for an upcoming season with average market and climate conditions; instead, they manage for adverse conditions (Rosenzweig and Binswanger, 1993). Thus, reducing uncertainties of seasonal climate forecasts may help farmers select more profitable farm management strategies. The fore knowledge therefore calls for re-opening of an enabling policy environment that effectively considers the

farmers perception in making policy options towards climate change. This becomes expedient given the farmers socio-economic characteristics that govern their environment. The fact remains that, even if "human" causes of climate change are controlled; the environment is still much bewildered with other natural processes leading to climate change (especially temperature and rainfall; humidity) and the aftermath effect on the agricultural economy.

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Frequency of Distribution of Interleukin 6 Gene 174G/C Polymorphism in obese Egyptian Cohort

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Abstract: Elevated IL6 level is documented in obesity. 174G/C polymorphism in the promoter area of IL6 gene may alter its expression or its effect. Aim: Finding the frequency of IL-6 174G/C polymorphism, its sex distribution and its association with obesity and dyslipidemia. Subjects and methods: This study included 74 apparently healthy subjects (45 men and 29 women). BMI was calculated. Lipid profile was assessed by specific colorimetric assays. Serum IL6 level was measured by ELISA. IL6 genotyping was done by PCR-restriction fragment length polymorphism. Results: According to IL6 polymorphism, subjects were classified into 3 groups; CC, GC and GG. 71.42% of the GG group and 68.97% of the GC group were male. Similarly, 78.57% of the GG group and 72.41% of the GC group were obese. No significant difference as regarding sex or BMI was found in the CC group. No significant difference was found among the 3 groups as regarding IL6 level. According to BMI subjects were further classified into Obese (more than 25Kg/m^2) and Non obese (less than 25Kg/m^2) .IL6 and triglyceride levels were higher in the obese group while HDL cholesterol was higher in the non-obese group. Both GG and GC genotypes showed significant positive correlation of IL6 with BMI (P=0.024 & P=0.012 respectively). Moreover, the GC group showed a significant positive correlation between IL6 and LDL cholesterol (p=0.022). CC genotypes didn't show any correlation of IL6 with either BMI or any measure of the lipid profile. Conclusion: The G containing alleles, GG & GC carriers are prevalent in male Egyptians which make them more vulnerable to obesity and its deleterious outcome than CC carriers.

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Keywords: Interleukin 6, Polymorphism, Obesity, Dyslipidemia.

1. Introduction

There is no doubt that the prevalence of obesity is high and continually growing all over the world reaching to the level of an epidemic or a pandemic disease[1]. Facing the health hazards and consequences of obesity is a big challenge especially for the developing countries. To overcome this problem, understanding the pathogenesis of obesity is mandatory and is the main target of a lot of researches in the recent time.

Dealing with the adipose tissues is changed in the recent years from a big junk for fat to a highly biologically active organ. It releases huge amounts of hormones and inflammatory cytokines. Hence, The obese patient is in a state of continuous sub-clinical inflammation[2].

One of these mediators is interleukin 6 (IL6). It has been discovered for the first time as an acute phase reactant promoting inflammation[3]. On the other hand, it exerts anti-inflammatory action through stimulation of the hypothalamic-pituitary-adrenal axis[4]. In these acute conditions, the main source of IL6 is the immune cells[5]. Another important source of IL6 production is the adipose tissue where IL6 was the second molecule proved to be secreted by fat cells[6]. The pleotropic nature of IL6 gave it the very wide range of actions all over the body. It has endocrine functions such as growth hormone secretion, inhibition of thyroid-stimulating hormone secretion, and reduction of serum cholesterol. It also stimulates LDL receptor gene expression[7]. It is involved in the pathogenesis of rheumatoid arthritis and osteoporosis. Furthermore, it is secreted during stress, steroid withdrawal syndrome and in traumatic states associated with the inappropriate secretion of vasopressin. IL6 may contribute to illness during aging and chronic stress[8].

In the last decade, several studies showed the elevated IL6 in obese subjects. However, these studies failed to explain the relationship between this peculiar molecule and its source i.e. the adipose tissue[9-11]. Furthermore, IL6 deficient mice develop obesity and its weight is reduced after IL6 adminstration[12]. Up till now, no explanation links these conflicting data together.

The IL6 gene consists of 5 exons and 4 introns located on the short arm of chromosome 7[13]. A genetic polymorphism is discovered at the 174

nucleotides upstream of the major transcription initiation site of the IL6 gene. According to the presence of either guanine or cytosine at this position two different IL6 alleles exist. These two different alleles give rise to three possible IL6 genotypes: GG, GC, and CC[14].

Previous studies on G (-174) C polymorphism proved that the expression and effects of IL6 differ according to the genotype polymorphism. For example; IL6 concentrations were lower in patients with systemic onset juvenile chronic arthritis who had the CC genotype[14]. In addition; plasma IL6 concentrations were higher in patients with abdominal aortic aneurysm who had only the CC genotype[15]. Other studies have indicated that the G (−174) C IL6 genotype does not affect plasma IL6 effect[16].

This study aimed to evaluate the frequency of IL6 gene polymorphism according to BMI and sex of the subject and to find out if certain IL6 polymorphism is related to obesity or lipid profile in these subjects.

Subjects and Methods

Subjects:

The study group included 74 subjects; 45 men and 29 women aged 21-68 years. Written informed consent was obtained from all subjects during the enrollment. The subjects were volunteer relatives to patients admitted to the internal medicine department, Menoufya University hospital. Subjects with acute inflammation, pregnancy, hypertension, Ischemic heart disease or diabetes mellitus were excluded from the study.

All studied subjects were submitted to full history taking, clinical examination and laboratory investigations.

Sampling:

Under complete aseptic conditions, 5 ml of venous blood were collected after 12 hour fasting & divided into two tubes as follows: Tube A, 1ml of blood collected in citrate (to prevent clotting and DNA degradation) for DNA extraction and kept immediately at -20 C°. Tube B, 4 ml of collected blood, left to clot serum was separated and used for immediate assay of lipid profile .The rest of the serum was kept at -20 C° for assay of IL-6.

Laboratory Methods:

Total cholesterol, triglyceride, and HDL-C concentrations were determined by using an enzymatic colorimetric assay on Synchron Cx9. LDL-c concentration was calculated according to the Friedewald equation (Friedewald W.T. et al., 1972).Serum interleukin concentration was measured by Kit was supplied by AviBion Human IL-6 ELISA Kit (Orgenium Laboratories). Intra-Assay-Precision of this test was <9.4%, and sensitivity was <2 pg/ml.

DNA analysis:

PCR-RFLP method was used to determine the distribution of genotype frequencies of the IL-6 C174G of the interleukin-6 gene. The DNA was isolated and purified by genomic DNA purification kit (GeneJET™ Genomic DNA Purification Kit, Fermentas International Inc., Canada). The PCR was performed on 10 ug DNA in 20 uL sterile D.W (2uL vol contain 1 ug DNA be added to the master mix),10x dream buffer, 0.2 mL dNTP, 200 U Taq polymerase and 0.1 µM of each primer (Fermentas International Inc., Canada), Interleukin 6 (IL-6 C174G) forward and reverse primers (Fermentas International Inc., Canada) as follows: for. 5'-TGA CTT CAG CTT TAC TCT TTG T-3'and rev. 5'-CTG ATT GGAAAC CTT ATT AAG-3', for 35 cycles (30 s at 94 C°, 30 s at 60 C°, 30 s at 72 C°). Each PCR cycle consisted of denaturation for 60 seconds at 94°C, annealing for 95 seconds at 55°C, and extension for 60 seconds at 72°C; followed by a final extension at 72°C for nine minutes. PCR products were digested using the restriction enzyme ***Sfa***NI (Fermentas International Inc., Canada). The digested samples were separated by electrophoresis on 3% agarose gel stained with ethidium bromide and visualized on a UV trans-illuminator. The presence of a single 198 bp band corresponds to the CC genotype; bands at 140 and 58 bp correspond to the GG genotype; and the presence of three bands corresponds to the GC genotype (Fig. 1).

Statistical analysis:

Frequency of distribution analysis was performed with a Chi2 square test. The significance level was set at 0.05 or less. All data analysis was performed using SPSS 11.0 software.

3. Results

According to the polymorphism; subjects were divided into 3 groups; CC, GC and GG. According to BMI subjects were further divided into obese group (more than 25Kg/m^2) and non-obese group less than 25Kg/m^2.

Frequency of distribution of IL6 gene polymorphism:

According to the sex, both GC and GG polymorphism showed a higher male predominance with a high statistical significance of 0.041 and 0.023 respectively. On the other hand, no significant difference was found between male and female in the CC group (Fig. 2).

According to BMI, a statistically significant higher number of obese subjects were found in both the GC (69%) and the GG (71.43%) groups. On the other hand, no significant difference was found between obese and non-obese subjects in the CC group (Fig. 3).

Comparison between all types of polymorphism:

No significant difference was found between the three groups as regarding age, BMI, IL6 level or the lipid profile (Table 1).

Correlations between serum IL-6 levels and both basal measures and biochemical results:

Pearson correlation analysis was done in each group separately. In the CC group IL6 levels didn't show any correlation with BMI or any measure of the lipid profile. Both GC and GG showed correlation of IL6 with BMI (Figure 4). Furthermore, in the GC group, IL6 correlated also with LDL cholesterol (Figure 5). None of the three groups showed correlation of IL6 with total cholesterol, HDL cholesterol or triglycerides (Table 2).

Comparison between the obese and non obese groups:

Both the obese and non obese groups were similar as regarding age, sex, total cholesterol and LDL cholesterol. IL6 and triglycerides levels were significantly higher while HDL cholesterol was significantly lower in the obese group (Table 3).

Table 1: Comparison between all types of polymorphism of the whole population.

P1: the difference between CC & GC P2: the difference between CC & GG P3: the difference between GC& GG

Parameter	Polymorphism			P Value		
	CC (N=17)	GC (N=29)	GG (N=28)	P1	P2	P3
Age (years)	38.47 ±	49.00 ± 13.72	30.01 ± 5.82	0.366	0.978	0.257
Sex (M/F)	5/12	20/9	20/8			
BMI (Kg/ m^2)	28.38 ± 5.15	30.24 ± 5.87	30.01±5.8	0.709	0.781	0.923
IL6(pg/ml)	2.12 ± 0.83	2.1 ± 0.95	2.11 ± 0.91	0.367	0.520	0.756
Total Cholesterol(mg/dl)	215 ± 41	240 ± 25	226.1 ± 35.43	0.133	0.619	0.300
LDL-C(mg/dl)	130 ± 26.55	138 ± 26.28	145.71 ± 30.41	0.796	0.572	0.360
HDL-C(mg/dl)	58.76 ± 22.6	51.17 ± 24.73	45.79±17.85	0.976	0.057	0.078
Triglycerides(mg/dl)	160 ± 55	164 ± 58	176.5 ± 60.42	0.453	0.554	0.906

Table 2: Correlations between serum IL-6 levels and both basal measures and biochemical results.

Parameter	**Interleukin 6 level**					
	CC		**GC**		**GG**	
	R	**P value**	**R**	**P value**	**R**	**P value**
BMI (Kg/ m^2)	0.314	0.219	**0.460**	**0.012**	**0.424**	**0.024**
LDL-C(mg/dl)	0.214	0.410	**0.424**	**0.022**	0.253	0.194
Total Cholesterol(mg/dl)	- 0.102	0.698	0.026	0.892	0.017	0.931
HDL-C(mg/dl)	- 0.161	0.517	-0.303	0.110	0.033	0.868
Triglycerides(mg/dl)	- 0.077	0.770	0.214	0.265	0.174	0.375

Table (3): Comparison between obese and non obese patients in the whole group

Parameter	Non obese (No=20) Mean ± SD	Obese (No=54) Mean ± SD	P Value
Age (years)	46.7 ± 15	44.72 ± 13	0.251
Sex (M/F)	34/20	11/9	0.063
BMI (Kg/ m^2)	22.3 ± 1.17	32.47 ± 3.89	0.000
IL-6 (pg/ml)	1.16 ± 0.87	2.28 ± 0.86	0.041
Total Cholesterol	217 ± 27	233 ± 35	0.637
LDLc(mg/dl)	107 ± 15	151 ± 26	0.097
HDLc(mg/dl)	74.75 ± 22	42 ± 14	0.006
Triglycerides (mg/dl)	89 ± 52	197 ± 21	0.000

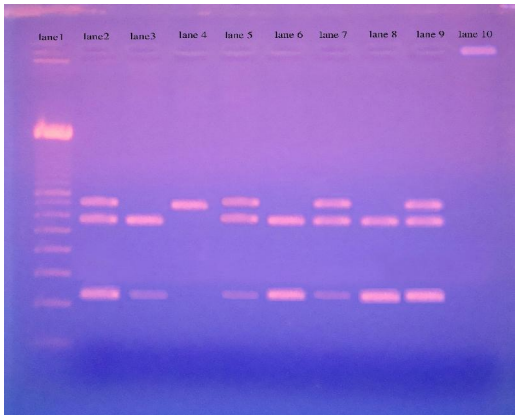

Figure (1): The three genotypes identified: lane 1 correspond to DNA (GeneRoler™ low range 25-700 bp) ladder; lane 2, 5 ,7 and 9 correspond to GC genotype (58,140 and 198 bp); lane 3,6 and 8 correspond to GG genotype (58 and 140 bp) and lane 4 correspond to CC genotype (198 bp).

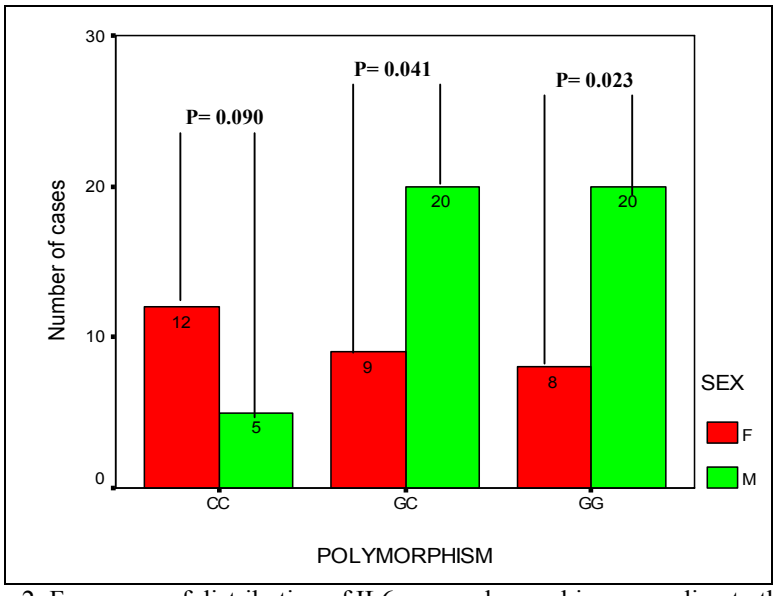

Figure 2: Frequency of distribution of IL6 gene polymorphism according to the sex

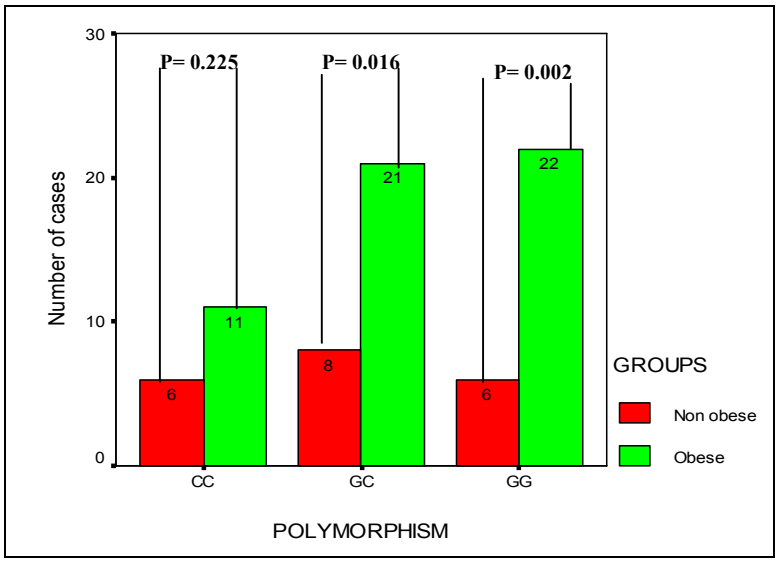

Figure 3: Frequency of distribution of IL6 gene polymorphism according to body weight

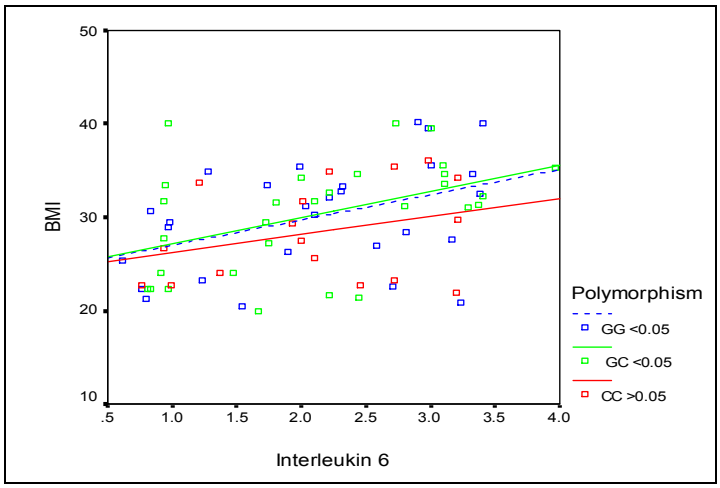

Figure 4: correlation between IL6 and Body Mass Index in different groups according to IL6 gene polymorphism.

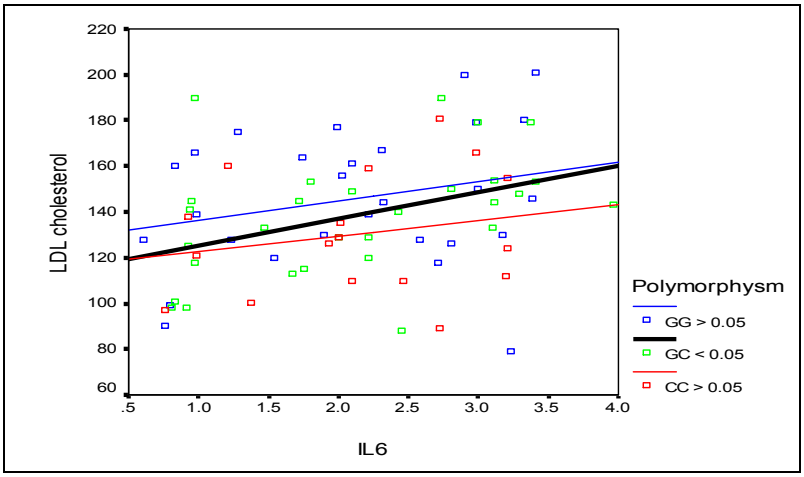

Figure 5: correlation between IL6 and LDL cholesterol in different groups according to IL6 gene polymorphism.

4. Discussion

The present work is a cross-sectional study on a group of apparently normal subjects. Its main purpose is to show the frequency of distribution of IL6 gene polymorphism according to the sex and its subsequent effect on obesity and lipid profile. In the GC and GG groups more than two thirds of the patients were male while there was no significant sex difference in the CC group. Interestingly, IL6 level correlate with BMI in the same two groups and not the CC group. Hence IL-6 (-174G>C) gene polymorphism; concerning GC and GG genotypes may be linked with male sex and obesity. This association was noted 3 years ago in elderly men by Strandberg et al., [17].

A striking finding, in this work, is the non significant difference in the distribution of the CC polymorphism between obese and non obese persons. On the other hand the GC and GG polymorphism showed a significantly higher distribution in obese subjects. 72.4% of the GC group and 78.57% of the GG group were obese.

With subgroup analysis, in the CC group, IL6 did not show any correlation with BMI or any measure of the lipid profile. On the other hand, in the GG group, IL6 showed a significant positive correlation with the BMI but not with the other measures of lipid profile. In the GC group, IL6 showed a significant positive correlation with both BMI and LDL cholesterol. This is in agreement with Fernandez-Real et al. who proved lipid abnormalities in subjects with the G allele [18]. Berthier et al., documented the correlation between IL6 and measures of obesity in patients with GC polymorphism[19]. The same results were confirmed in the EPIC-Potsdam study on 334 obese subjects[20]. However, Kubaszek et al., reached to a different result and linked the CC genotype with obesity and reduced energy expenditure [21].

The deleterious effect of the G allele was noticed in several studies. Bennermo et al., linked the GC polymorphism with inflammation and speculated its central role in the development of several diseases [22]. In the WOSCOPS study on Scottish patients, the G allele carriers had a higher risk for coronary artery disease than the C carriers[23]. Kocierz et al., in another recent study noticed that kidney transplant failure was associated with the GG and GC genotypes compared with the CC genotype [24]. Kayaalti et al., documented the advantageous effect of the C allele on the life span of Turkish people over the G allele [25]. Rheumatoid arthritis patients with the CC genotyping are resistant to treatment with Rituximab, an anti CD20 selective anti-inflammatory drug, confirming the absence of association between the C allele and inflammation [26].

Nevertheless, several studies failed to reach to a significant association between IL6 polymorphism and coronary artery disease [27] or diabetes mellitus [28].

Although there was no significant difference among all genotypes as regarding the IL6 level, there was association of the G allele only with obesity and elevated LDL cholesterol. This may refer to that the effect of gene polymorphism may be through its effect on other pathways rather than IL6 level itself. This is in agreement with Henningsson et al., who documented the absence of the association of IL6 genotyping and its plasma level [29]. Okada et al., proved the pivotal role of an IL6 locus in the regulation of serum CRP levels and inflammatory pathways [30]. Cardellini et al., showed that patients with GG genotype of the IL-6 gene have reduced insulin sensitivity which is a risk factor for a lot of deleterious vascular disorders [31].

Similar to the present result, different studies have documented IL6 elevation in obese subjects [32-34]. These studies, however, failed to reach to a consistent relation between IL6 and obesity. One decade ago, Roytblat and his co-workers tried to explain these opposing results. They suggested the complex etiology of obesity, diet and physical activity levels as factors affecting the relationship between IL6 and obesity. Moreover, this relationship may be additionally influenced by environmental, socioeconomic, and behavioral factors[32]. IL6 gene polymorphism may be a hidden factor at that time that may partially help to identify the heterogeneous results in the studies.

Conclusions:

IL6 is elevated in obese persons. G containing alleles are related to obesity and may be to its deleterious outcome while the CC genotype is not related to obesity. The effect of 174G/C polymorphism on obesity is not through its effect on serum IL6 level. Therefore 174G/C polymorphism in any future study on obese subjects should be considered. Dyslipidemia could be a mechanism promoting the deleterious effect of IL6. Other mechanisms, however, should be searched for.

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BACTERIOLOGICAL QUALITY ASSESSMENT OF WATER HAWKED IN SOME PARTS OF MUBI METROPOLIS, ADAMAWA STATE, NIGERIA

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Abstract: Investigation of bacteriological quality of drinking water hawked around five different areas in some parts of Mubi town in Adamawa state, Nigeria was conducted, using most probable number (MPN) technique and standard plate count methods. Two samples were collected from each site (one at the source and the other from hawkers at the point of delivery to house holds) which was repeated weekly for a period of one month. Mesophilic counts for the samples at the point of collection had values> 10^3 cfu/ml. whereas only two samples (Shuware and Lokuwa at the point of delivery) showed values = 10^3 cfu/ml. However, neither at the source nor at the point of delivery showed MPN values within acceptable range of <1O/IOOml. Therefore, the findings recommend improvement in sanitary quality at the source and enlighten the hawkers on measures that reduce microbial contamination during handling.
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Key words: Bacteriology; water; quality; hawkers; Mubi

1. Introduction

Water makes life possible on earth for all living organisms. It is next to air in importance for human existence. The importance of water to life on earth particularly human becomes clearer when one considers its role, or usefulness in various aspects of human endeavours. Even though a lot of resources are being spent for the supply of clean and potable drinking water all over the world, achievement of this aim is still facing serious plight in Nigeria.

Currently, the emergence and proliferation of water vendors using carts with multiple jerry cans in Mubi town is an issue of concern in public health sectors especially with the common incidence of enteric diseases like typhoid fever, cholera and dysentery. In recent months, there has been an out break of cholera like disease in Mubi town where 70 — 80 people were reported to have died. This inspired this study to investigate more on microbiological quality of water hawked within this part of Adamawa state.

Idakwo and Abu (2004) reported that a wide variety of microorganisms pathogenic to human beings are transmitted through contaminated water. According to WHO (l982) some 300,000 people die every day from water related diseases like typhoid and paratyphoid fevers, cholera, bacillary dysentery and gastroenteritis.

Therefore, in order to substantiate the nation's effort to provide and ensure the provision of safe drinking water, this study was conducted with the aim of ascertaining the bacteriological quality of water supplied in jerry cans by vendors in some parts of Mubi town and to recommend some possible measures of ensuring its portability.

2.0 Materials and Methods

2.1 Sampling Sites: A survey was conducted on areas having plights of water scarcity in Mubi town. Emphases were made to places that served as commercial and/or public water sources, and these places were identified. Site where hawkers collect and sell water in jerry cans were chosen for the sampling. The sites are, Arahan Kunu, Lokuwa, Kolere, Shuware and Wuro Patuji.

2.2 Sample Collection: Sample collections were carried out in two forms. One from the main source (reservoir) and the other directly from the jerry cans of the hawkers at the point of delivery to a house hold. This was repeated four times a month on weekly basis. For each sampling sites, samples were collected in a sterile *250m1* capacity dark brown glass sampling bottle (Cheesebrough, 2000) in each case. All samples were transported to the laboratory and kept under refrigeration for analyses.

2.3 Detection and Enumeration of Coliforms: This was carried out according to the method described by Harold (1998). Each sample was inoculated into 3 sets of tubes as follows:

First, l0ml into a tube containing 40m1 of lactose broth, usually designated as double strength lactose broth (DSLB) with Durham tubes, then 1.0ml of the 20m1 of lactose broth, usually designated as single strength lactose broth (SSLB) with durham tubes, and then O.lml inoculated into three tubes each containing 20m1 of lactose broth, usually designated

as single strength lactose broth (SSLB) with durham tubes. The tubes were incubated at 35^0C for 24 — 48 hrs. Following incubation tubes showing gas production were counted and compared with MPN table adapted from APHA (1998) for the determination of most probable number (MPN) of coliforms per 100mls of water.

A loopful of broth from gas positive tubes was seeded on to eosin methylene blue (EMB Antec UK) agar plate and incubated at *35°C* for 24 hours. The plates were observed after 24 hours for the presence of bluish black colonies with green metallic sheen which confirmed the presence of Coliform bacteria.

2.4 Standard plate count

This was carried out according to FAO (1979). In this method; 1ml of sample was transferred into a test tube containing 9.0ml of sterile distilled water and the tube labeled 1: 10. From this tube 1.0ml was transferred after agitation into another tube containing 9.0 ml of sterile distilled water and labeled 1: 100. This was also agitated and the procedure was repeated up to 1: 1000. Using sterile pipette 1.0ml of inoculum was transferred from dilution tubes into appropriately labeled duplicate Petri dishes. This was followed by pouring a cooled molten nutrient agar (oxoid). The dishes were gently rocked, allowed to solidify and incubated at 37^oC for 24hours. After 24 hours incubation, plates containing 30 -300 colonies were counted and the number obtained multiplied by the reciprocal of the dilution factor to get the actual number of organisms. The results were finally expressed in colony forming unit per ml (cfu/ml) of the sample.

Table 1: Mean Count of The Bacterial Load obtained from different water sources

Sampling Site	**Mean Mesophilic Count (cfu/ml)**	
	Water source	Jerry Cans
Arahan Kunu	3.00×10^4	4.20×10^5
Wuro Patuji	2.91×10^3	3.23×10^4
Kolere	2.00×10^3	2.25×10^4
Shuware	2.22×10^3	3.11×10^3
Lokuwa	1.00×10^3	3.67×10^3

Key: S= Source and J= Jericans. E.g AS= Arahan kunu source, AS= Arahan kunu jericans.

2.5 Biochemical characterization of *E. coli*

Indole Test: Three loopfuls of the material from colonies formed were inoculated into a bijou bottle containing 3 ml of sterile trypton water and incubated at 37^0C for 48 hours. This was followed by the addition of 0.5 ml of Kovac's reagent. Appearance of red ring on the surface of the medium is positive for indole production which was confirmed for *E. coli* (Cheesebrough, 2000).

2.6 Methyl red test:

Tube of methyl red - Voges Proskauer (MRVP) broth was inoculated with three loopfuls from the suspected colony of *E. Coli*_and incubated at 37^oC for 3 days. This was followed by the addition of few drops of methyl red indicator. Appearance of red colour confirmed acid fermentation (positive for *E. coli*) (Cheesebrough, 2000).

3. Results

Arahan kunu, Wuro patuji, Kolere, Shuware and Lokuwa wards obtained their drinking water from commercial water source hawked by truck pushes. Sixty percent (60 %) of these supplies had bacterial count of 1 0 cfu/ml and about 40 % had bacterial count of = 10^4 cfu/ml. The MPN value ranged between 21— 120 per 100ml with lowest at Lokuwa water source (Table 1) corresponding to the lowest mesophilic count. *E. Coli* was detected in 80% of the samples.

Table 2: Most Probable Number of Coliforms Present In Water Samples From The Various Sampling Location in Mubi

Numbers of gas positive tubes				**MPN index per 100ml**
Sample	3 of 10 ml each	3 of 1 ml each	3 of 0.lml each	
AS	3	1	0	43
AJ	3	1	2	120
WS	3	1	0	43
WJ	3	1	1	75
KS	2	2	1	28
KJ	3	0	0	39
SS	2	2	1	28
SJ	3	0	1	39
LS	2	2	0	21
LJ	3	0	1	39

4. Discussion

The result for bacteriological assessment of water hawked in the selected areas of Mubi town indicated contamination at both the source (reservoir) and the point of delivery to the consumers. The counts at the sampling sites are however higher at the point of delivery to the consumers than at the source. This suggests that the increase in microbial load might be as a result of poor handling of the water or the use of unclean containers (Jerry cans). This result agrees with the work of Muktar et al., (2007) who reported that 90 % of water hawked in some selected areas in Kano had bacterial count of 10^4 cfu/ml and MPN values ranged between 9 and > 180 per 100ml. This result is also in conformity with the work of Muktar and Oyeyi (2005), who reported that some of the raw water sources from some open wells in Kano had Coliforms of up to 15 MPN/l00ml.

Furthermore, the result obtained from the standard plate count shows conspicuously that people living around these areas of study namely: Arahan kunu, Wuro patuji, Kolere, Shuware, and Lokuwa are exposed to risk of contracting infection by the organisms isolated, which might lead to an outbreak of gastroenteritis and other enteric diseases.

The distribution of such important commodity (water) in the hands of illiterate members of the society who are habitually dirty and always unhygienic may be responsible for the introduction of microbes into the water, because most of the hawkers don't wash their jerry can thoroughly and regularly.

The entire study area has been known with long history of prominent water scarcity. The inability of the government to provide potable drinking water had contributed immensely to the water scarcity, creating more public health problems. People depend heavily on truck pushers who sell water some of which were obtained from doubtful sources.

5. Conclusion and Recommendations

In conclusion, people living in the study areas are at high risk since the work has shown that there is contamination of water sold by hawkers in the areas by feacal Coliform *(E. Coli).* Hence the following recommendations are suggested.

i. Regulatory agencies (both governmental and nongovernmental) should intensify their efforts towards providing clean and portable water to the public.

ii. Water hawkers should maintain personal hygiene and should always use clean and leak proof containers for their business.

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Designing Reinforced Concrete Frames with Earthquake Damage Control

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Abstract: Controlling the amount of structural damage is one of the most important issues in new design methods such as performance-based design. The purpose of present research is to present a new design method with damage control for reinforced concrete bending frames. For this purpose, at first a static damage standard is developed and then suggested method is applied to design a 7 store frame. Then in order to assess suggested method accuracy, non-elastic damage analysis is performed on mentioned frame. The results show that suggested method is effective on designing reinforced concrete bending frames, with damage control.
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Keywords: Damage, performance-based design, Bending frame, Reinforced concrete, non-elastic analysis.

1. Introduction

Usually and traditionally, the main issue in earthquake design of structures is to preserve residents' life with the hope that structural damage after earthquake can be repaired. California earthquake in recent years showed that the amounts of structural damages in these earthquakes were unacceptable and cost needed for repairing structures were very high. Attempts to modify design methods and design regulations criteria finally resulted in developing new method named "performance-based design". The main subject in performance design category is to control the amounts of structural damages under earthquake. For this purpose, according to performance standards, a relation between introduced performance levels and the amounts of expected structural damages has been created [1]. Introduced performance levels in standards, include four levels which are usable level, immediate exploitation, life safety and preventing collapse respectively. [2, 3]

The potential damage of an earthquake on structures is one of the base discussions in earthquake engineering. A reliable scale of earthquakes damage potential can have many applications in new structures analysis and design and also present available buildings seismic assessment [4]. Earthquake parameters such as maximum acceleration and elastic response spectrum although are many important but can't provide reliable standards of an earthquake's damage potential. One way for measurement of the amount of structural damage is to use damage indices [5-9]. A damage index is stated based on combination of many structural deformations which cause damage and also the amount of damage which is caused because of cyclic loading repetition. Until now, different damage indices have been introduced but none of them are applicable widely. Another way to estimate degree of structural damage is to estimate the relation between damage and relative storey's movement. Relative storey's movement is one of the initial parameters in assessment of structure performance [10, 11] and has been broadly taken into consideration as a main parameter in measurement of buildings plastic deformations [12, 13]. Of course some researches show that this parameter is an unsuitable and insufficient standard in structural damage control. Since relative movement standard is an insufficient parameter a structure performance (or damage) control and on the other hand since damage index assessment include dynamic hysteresis energy calculation which is so time-consuming and a complicated process, so in this research an effective and applicable standard for estimation of reinforced concrete bending frames and by using of push over Analysis results is developed. The main purpose of this research is development and extension of a new method for designing reinforced concrete bending frames, with damage control, based on this research suggested damage standard. For this purpose, initially functions for estimation of structural damage are obtained by using of push over analysis and then suggested method is used for designing a 7 story reinforced concrete frame under 7 earthquake records. Then, in order to assess suggested method accuracy, non-elastic damage analysis is done. Results show that suggested method can be very efficient and applicable in designing reinforced concrete bending frames, with damage control.

2. Dynamic Damage Index

In this research the amount of structural damage in dynamic analysis is calculated by using of Park and any damage index. These researchers' suggested index is a compound index based on deformation and wasted energy amount in the

structure [7]. These researchers have calibrated their suggested damage index by observing physical damage on several samples. Appropriateness of real damage to index values suggested by these researchers has been presented in table 1.

Table 1- Details of Damage Proportionate to Park and Ang index

Damage degree	External appearance	Damage index	Building situation
Collapse	Appearance or total collapse of building	>1	Demolished
Intense	Concrete wide crushing, appearing ricochet amateurs	0.4-1.0	Can't be repaired
Moderate	Large and extensive cracks, concrete delaminate in weaker members	0.25-0.4	Repairable
Little	Small cracks, local crushing of concrete in columns	0.1-0.25	Repairable
Slight	Appearing sporadic cracks	<0.1	Repairable

This index value is equal to 0 when structure remains elastic (i.e. it hasn't happened any damage) and will be equal to when it happen so damages that structure has potential to break. Other performance states such as immediate exploitation and life safety and so on are defined by consideration of a range between 0 and 1. In order to determine Park index range corresponding to performance levels, by refer to performance standards such as ATC40 [16] and FEMA273 [2] and also table 1, we can correspond usable performance levels, immediate exploitation, life safety and collapse prevention to damage degrees of without damage (slight), little damage, moderate damage and severe damage of Park index respectively.

3. Static Damage Index

In this research energy damage index has been suggested in order to measure the amount of structural damage by using of push over analysis results. Using wasted energy by structure, has been applied in many researches in order to determine the amount of structural damage most of which have been based on dynamic analysis. Kato and Akiama took into consideration the wasted accumulated energy by hysteresis attenuation as an acceptable index for estimation of structural damage [17]. Zhang et al. used input energy and structure plastic energy for determining structural damage [18] and so on. Referring to energy balance equation for none-linear system, under earthquake:

Equation (1, 2)

Or

KE+DE+SE+PE=IE

In which KE is kinetic energy, DE is attenuation energy, SE is strain energy, PE is plastic energy and IE is the energy into the system. As you can see, the energy into the system is wasted through 4 mechanisms. In general stare, kinetic and strain mechanisms undertake little portion of the amount of energy wasted by structure and main amount of energy is wasted by attenuation and yield energy. In these two mechanisms also the more structure enters non-linear phase, portion of yield energy is more than attenuation energy which indicates non-elastic mechanisms are slower than elastic systems. The purpose of this section is to provide an index based on energy absorbed by structure in push over analysis. Since, capacity curve resulted from push over analysis is structure Hysteresis push-rings, then we can say that the area below the curve in performance point, introduces energy absorbed by structure in its largest Hysteresis ring under certain earthquake which often has great portion of energy absorbed by structure. This index is calculated by following relationship:

Equation (3)

In which E_{pp} is the area below capacity curve in performance point, E_{ip} is the area below capacity curve in a point corresponding to entering structure into non-linear phase and E_{fp} is the area below capacity curve in a point corresponding to section final capacity which are explained then. In order to calculate E_{pp} area, at first is calculated by using of numerical integration methods of area below capacity curve in performance point and then with respect to existing relations in ATC40 [2] yield point is determined. Afterwards, using below relationship, which is available in reference [2], concerning ¼ of structure Hysteresis ring is calculated:

Equation (4)

In which d_p and a_p are yield point coordinates and d_y and a_y are coordinates of performance point. In definition of this index, the primary point which is entry of structure to non-linear phase and is considered as beginning of structural damage, a point of capacity curve has been considered correspond to the first crack of structural members and damage index value in this point is equal to 0 and the more structural behavior enter non-linear, structural elements are more damaged and this index value will be more, until eventually in structure final capacity point, value of this index will become 1. In order to determine structure final capacity various definitions have been propounded, but in this research, the structure final capacity point is considered a point form structure capacity curve in which by slight increase in lateral force, structure encounter a very large sudden deformation compared to the previous step and in other words jump occur in

structure capacity curve. Steps for calculating E_{fp} also is similar to E_{pp}. E_{ip} is area below capacity curve in first crack point. It should be noted that hereafter this index will be named energy damage index.

4. Designing Frames

In this section in order to assess damage indices introduced in previous sections, 14 reinforced concrete frames were considered so that include a large number of storeys and spans [15]. Four-span frames include 5, 8, 12, 15 storeys, five-span frames include 4, 6, 2, 8 and 10 storeys and two-span frames include 1, 3, 5, 7 and 9 storeys. The height of all storeys were equal to 3.2 and length of spans also were equal to 4 m. when modeling these frames it's assumed that all frames are placed in stone beds and have been loaded for region with moderate risk according to standard 2800 and designed according to ABA regulations (In designing these frames, all criteria such as lateral deformation restriction in regulations 2800 has been considered). Frames have 4 m loader width and in all storeys have dead load of 760 kg/2m and live load of 200 kg/m^2. According to standard, importance of frames has been assumed moderately 2800. In process of analysis and design of these frames, characteristic strength of concrete has been assumed equal to 30 Mp, concrete elasticity modules 27386 Mp, strain correspond to maximum strength of concrete 0.002, concrete final strain 0.003, flow resistance of steel 300 Mp and elasticity modules of steel has been assumed 200000 Mp.

5. Earthquakes Calibration

In this research, 7 earthquake records from records set available in FEMA440 [19] had distance from fault and were selected proportionate to geotechnical properties of stone bed and measured according to 2800 regulations criteria so that spectrum of average obtained from them in range of 0.03 to 2.4 s (which based on regulations 2800 [17] is important range for frames designed in this research (the range between 0.2T and 1.5T)) has minimum difference with 1.4 times of spectrum of regulation 2800. Properties concerned with these records and average spectrum obtained from them is available in reference [20].

6. Damage Analysis

The purpose of this section is measurement of energy damage index. For this purpose, after structures' modeling, push over and non-linear dynamic analyses were performed on them and energy damage index values in push over analysis and Park damage index values in dynamic analysis were calculated. In order to calculate damage indices in push over analysis initially structure performance point should be determined and then these indices values should be calculated in structure performance point. In order to determine performance points, capacity spectrum method according to descriptions presented in reference [21] has been used. Afterwards, in order to determine the relation between stated indices, 5 performance points were calculated for each frame which were related to records average response spectrum and spectrums which are 1.5, 2, 2.5 and 3 times this spectrum respectively. Then damage indices values in these points were calculated and after that in order to calculate damage indices in dynamic state, for each spectrum, existing records were converted to criterion and nonlinear dynamic analysis of frames were done and according to each calculated damage in performance points in push over analysis, by averaging results related to 7 selected earthquakes, damage related to non-linear dynamic analysis were calculated, which concerned results will be presented. It's notable that in figure 1, triangle shaped points are relevant to damage correspond to average spectrum and correspond to designed earthquake of regulations 2800 and circle-shaped points are correspond to 1.5 times average spectrum, which approximately can be correspond to risk level M.E. in ATC40, which indicates 5% contingency during 50 years. Thus in order to conservative estimation of damages at this level, the maximum limit for this estimation i.e. 1.5 times average spectrum has been selected) and other points have been specified by rhombic. Hereafter in this research we mention these points by naming the shape.

7. The Relationship between Indices

In this section, we have compared values of Park and Ang damage index in dynamic analysis to values of energy damage index which have been calculated in performance points according to section 3. As you can see in figure 1, at the beginning of this chart, Park and Ang damage index values are higher and as we move toward the end of chart, this difference is reduced and by referring to presented descriptions in section 3 we can say that this issue is because of increasing Hysteresis energy share of wasted energy by structure and more entrance of structure into non-linear phase. Moreover, the energy damage index values average is 0.4 which is very close to Park damage index average. Other notable material in this chart is that energy damage index variations range for circle-shaped and triangle-shaped points is 0.027 to 0.176 and 0.11 to 0.36. As you can observe in figure 1, presented energy damage index in this research, has scattering points proportional to Park and Ang damage index in dynamic analysis and since this index is general and has simple calculation

steps, so using it in recognition of structural damage can be very effective. In order to increase the accuracy in presented relationships between these indices, there have been specified various ranges of damage which respectively are little damage range (dynamic damage: 0-0.26), moderate damage (Dynamic damage: 0.26-0.52) and intense damage range (dynamic damage: 0.52-1.0). In each of these ranges, by interpolation between these index values, relations have been presented as follows by using of which we can obtain Park damage index values from results of push over analysis and energy damage index values (for little to intense damage ranges respectively):

(5) $DI_s = 0.255DI_D + 0.052$

(6) $DI_S = 1.237DI_D - 0.187$

(7) $DI_S = 1.8DI_D - 0.448$

Which in these relations, DI_D is Park damage index in dynamic analysis and DI_S is static damage index in push over analysis. Although by using of equations 5 to 7 we can calculate Park index values with good accuracy, but since the purpose of designing is to provide a safe pattern, so equations 8 to 10, which are related to conservative pattern in little to intense damage ranges, is presented as follows:

(8) $DI_S = 0.255DI_D + 0.05$

(9) $DI_S = 1.237DI_D - 0.292$

(10) $DI_S = 1.8DI_D - 0.62$

8. Suggested Design Method

In this research suggested method, it's considered that the structure which has been designed based on traditional methods for regulations design spectrum should be able to tolerate a more intense earthquake so that the amount of damage on that structure can be controlled. For this purpose, initially the structure typically is designed for regulations design spectrum and then energy damage index value for a more intense spectrum is calculated by performing push over analysis and by using of presented equations, its corresponding Park index value is calculated. After that, calculated damage value is compared to range considered by designer. Now, if damage is more than expected range, then structure should be strengthened and again static damage be calculated and again be compared to considered range. These try and error operations should be repeated so that finally designer's viewpoint about damage is met. The important point is that what members should be strengthened. For this purpose and in order to achieve acceptable pattern by spending minimum cost, in each repetition, on structure performance point the amount of wasted energy in structure various members is calculated and members which have absorbed most energy, are selected for strengthening. It's notable that in each damage range, 3 lines have been provided which equations 5 to 7 are related to acceptable pattern with minimum safety and equations 8 to 10 are related to very conservative pattern and design band between these lines can be considered in design. By more approaching equations 8 to 10, safety becomes more. Thus a pattern is considered acceptable when its calculated damage is in range of values obtained from equations of optimized pattern and conservative pattern.

9. Application of Suggested Design Method for a storeys Frame

In this section a 7-storeys and 3-spans reinforced bending frame is designed. This design example is different from frames considered to develop indices relations in section 7. This frame initially is designed based on spectrum of standard design 2800 and then is controlled for considered damage level. Considered properties for designing is according to section 4. Lateral load distribution is proportionate to exponential distribution, according to FEMA273. Strengthened spectrum is considered 1.3 times average spectrum (figure 2) (This spectrum is described more in section 10). The amount of dynamic damage is 0.4 which corresponds to live safety upper bound (according to table 1). This damage bound is placed in average damage range in figure 1 and thus equations 6 and 9 are used in order to determine acceptable damage index values corresponding to this dynamic damage. By replacing 0.4 in these equations static damage range will be 0.2 to 0.31. by performing push over analysis and calculating energy damage index, it's observed that energy index value is obtain 0.378 which is out of acceptable range and indicates that initial design is unacceptable for live safety damage level against strengthened spectrum. After that, by strengthening some members which have absorbed more energy, by increasing studs and repeating try and error three times, finally the amount of static damage was reduced to 0.26 which is placed in acceptable range. Spectrum and capacity and performance points of initial pattern and final pattern have been shown in figure 3.

10. Assessment of Suggested Method

In this section in order to assess effectiveness of suggested method, non-linear dynamic analysis is performed on mentioned frame. For this purpose, 7 earthquake records which properties have been listed in table 2, has been considered. These records have been considered so that their spectrum is placed in minimum distance from spectrum of standard design 2800. Average

spectrum of these earthquakes, standard 2800 spectrum and strengthened spectrum of them have been shown in figure 2. After that, non-linear dynamic analysis was performed for records corresponding to average spectrum on initial structure (not-strengthened) and Park damage index values were calculated and finally damage average of these earthquakes was obtained 0.48 which was out of acceptable dynamic damage i.e. less than 0.4. Then by performing similar procedure for final frame (strengthened) by using of records corresponding to strengthened spectrum (1.3 times average spectrum) again damage average for these earthquakes was obtain 0.346 (damage values of each of earthquakes in these two analysis, have been shown in figure 4).with respect to results, it's observed that initial structure against strengthened spectrum hasn't had desirable performance for live safety level and the amount of its damage has been more than acceptable value, but strengthened structure by using of research suggested method, has suitable amount of damage and by comparison between its amount of damage and bound dynamic damage value (0.4) it's observed that there is about 13% difference which indicates that suggested method has significant effect on assessment and anticipation of the amount of structural damage.

Table 2: Properties of selected earthquakes

Earthquake number	Record	Station	Component (deg)	PGA(g)
1	Loma Prieta	58151	90	0.092
2	Loma Prieta	58151	0	0.078
3	Loma Prieta	58539	205	0.105
4	Landers	21081	0	0.115
5	Landers	21081	90	0.146
6	Santa Barbara	283	222	0.203
7	Northrid ge	90019	180	0.256

11. Conclusion

In this research a new suggested method for designing reinforced concrete bending frames, with damage control, was developed by using of which we can place damage in designer considered range. To provide this method, a static damage standard was developed based on energy concept. The results of comparison between suggested damage index and Park index in non-linear dynamic analysis showed that introduced index scattering is proportionate to Park index. Then, in order to design structures a band named "design band" was considered. Eventually, suggested design method was used to design a 7-storeys 3-spans frame and results obtained from assessment of this standard through performing non-linear dynamic analysis showed that suggested method has desirable quality.

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Mechanical Properties of Acrylonitrile butadiene/ Ethylene Propylene Diene Monomer Blends: Effects of Blend Ratio and Filler Addition

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Abstract: Blends based on acrtlonitrile butadiene rubber (NBR) and ethylene propylene diene monomer (EPDM) rubber was prepared with different fast extrusion furnace black (FEF) concentrations. The effect of blend ratio and FEF contents on the mechanical properties, such as stress-strain behavior, tensile strength and elongation at break have been investigated. Tensile and elongation at break showed synergism for the blend containing zero NBR/100EPDM and 80 phr FEF-content. The experimental data have been compared with the relevant theoretical models.
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Keywords: NBR; EPDM; Rubber blend; mechanical properties

1. Introduction

There is great industrial interest in polymer blends since blending is a relatively simple way to create material with significantly improved properties. The properties of polymer blends are closely related to the number, shape and morphology of the dispersed phase particles [1-6]. Fillers, either powders or fibers, are introduced into a broad range of polymers to modify their mechanical properties, thus giving them new application and commercial value [7]. In recent years, the production of elastomeric blends has markedly increased, due to their well- balanced physical and mechanical properties, easy process ability and relatively low cost. Blending of two or more types of polymers is a very useful technique for the preparation and development of materials with properties superior to those of individual constituents [8]. Mechanical properties of blends are mainly determined by the component properties, phase morphology and adhesion between the phases. The phase morphology is dependent on processing factors such as type of mixer, blending rate, blending temperature, component rheology, interfacial tension and cross-linking agent.

Particulate fillers can increase the strength of an amorphous rubber more than 10-fold [9]. For a filler to cause significant reinforcement, it must possess high specific surface area, i.e., the particles must be small, less than 1 μm in size [10]. Small particles have large surface area to interact with the rubber and close particle-to-particle spacing in the compound. Two types of fillers that are most effective for reinforcing rubber are carbon black and silica. They can be produced with a primary size as small as 10 nm, corresponding to a surface area if a few hundred m^2/ gm of filler. The reinforcement of rubber properties by the incorporation of carbon black is due to the presence of active polar groups such as phenol, carboxyl, quinone and lactones on the carbon black surfaces [11- 13]. These polar groups on the carbon black surfaces interact with rubber and the interaction is higher with polar rubbers than hydrocarbon rubbers, which is due to polar- polar interaction [14]. The degree of reinforcement of the filled rubber depend mainly on the filler concentration and principal properties of carbon black such as particle size, surface area, aggregate structure and its distribution in rubber matrix as well as rubber-filler interaction [15].

Blends based on non-polar ethylene-propylene-diene monomer (EPDM) and polar acrylonitrile butadiene rubber (NBR) was prepared and FEF carbon black was introduced through these blends. The interaction between the hydrocarbon rubber and carbon black can be improved by the introduction of polar groups in the rubber through blending or adding some particular additives [16].

This paper describes recent investigations that carried out on the mechanical properties of NBR/EPDM blend filled by (fast extrusion furnace) FEF carbon black. The degree of crooslinking density for prepared compounds was determined. Mechanical properties of an elastomer depend strongly on crosslink density. Modulus increase monotonically with increasing crosslink density and the material becomes more elastic, or stated alternatively, less hysteretic. Fracture properties, such as tear and tensile strength, pass through a maximum as crooslinking is increased. An attempt

has been also made to correlate the mechanical properties with the existing theoretical models.

2. Experimental

2.1 Materials

Ethylene-propylene-diene monomer EPDM (EP35) had propylene content 43wt% and Acrylonitrile butadiene co-polymer rubber (NBR, Nipol) were supplied by Japan Synthetic Rubber Co., Ltd. Fast extrusion furnace FEF N550 carbon black CB, cure activators (zinc oxide and stearic acid) and curatives system based on sulphur were supplied by Transport and Engineering Co. (TRENCO), Alex., Egypt. The basic characteristics of EPDM, NBR and FEF are given in Table (1).

Table (1): Rubbers and carbon black characteristics

Material	Parameter	
Ethylene-propylene-diene monomer (EPDM)	Propylene content (%)	43
	Density (g/ cc)	0.86
	Mooney viscosity (ML_1+4; 100^{0}C)	83
	Volatile material (%)	0.5
	Ashes (%)	0.2
Acrylonitrile butadiene rubber (NBR, Nipol)	Acrylonitrile content (%)	33
	Density (g/ cc)	0.98
	Mooney viscosity (ML_1+4; 100^{0}C)	77.5
	Volatile material (%)	0.75
	Ashes (%)	0.5
Carbon black FEF (N550)	Average Particle Diameter (nm)	43
	N_2 Surface area (m^2/g)	42
	Iodine adsorption (mg/g)	44
	DBP[a] absorption (cm^3/100g)	115
	Ash content (%)	0.2
	Pour Density (g/cc)	0.38

a) Dibutyl Phthalate.

2.2 Sample preparation

EPDM / NBR conductive blends were prepared by the master batch technique. The blend compositions are [0/100, 75/25, 50/50, 25/75 and 100/0 respectively. Rubber blends with a different blend ratios of NBR and EPDM were prepared by mastication of each type then mixing for a period of 20 min. with different ingredients in phr (part per hundred parts of rubber by weight). Compounding was done on two – roll mixing mill (Friction ratio 1:4), according to ASTMD 15-627. After rubber mastication, the compound ingredients such as activators, FEF carbon black, processing oil, accelerators and sulpher were then added constituents in ratios as shown in Table (2). After compounding, the stocks were left for 24 h to mature. It was then cured into sheets of 2mm thick using hot press at 5 MPa pressure and heating temperature 145± 2^{0}C for 20 min. To insure reproducibility, the samples were conditioned at 70°C for 20 days [17].

Table (2): Composition of NBR- EPDM of different blend ratios:

Ingredients (phr)	NBR/EPDM: 100/ 0	NBR/EPDM: 75/ 25	NBR/EPDM: 50/ 50	NBR/EPDM: 25/ 75	NBR/EPDM: 0/ 100
EPDM	0	25	50	75	100
NBR	100	75	50	25	0
Stearic acid	2				
ZnO	5				
Oil	10				
FEF	0, 10, 40, 50 and 80				
MBTS[a]	2				
P N[b]	1				
Sulfur	2				

a) Dibenthazyl disulfide
b) Phynyl- - napthylamine

2. 3 Mechanical property measurements

The mechanical tests were carried out at 27^{0}C by using a home- made universal testing machine with a crosshead speed of 500 mm/min. Rubber composite samples were cut with a die into dog-bone shape of initial dimensions 40 mm in length, 4 mm in width and 2 mm in thickness. The recorded value for each mechanical parameter is the average of three measurements.

3. Results and Discussion

3.1 Stress- strain behavior

The influence of filler content on the mechanical properties of reinforced-elastomers has been extensively reported in the literature [18]. In this work, NBR/EPDM different blend ratios were reinforced with FEF black. The degree of reinforcement usually increases with filler loading. The degree of reinforcement will depend on the extent of polymer filler interaction and the degree of wetting of filler particles by the polymer matrix [19]. The stress- strain curves of different blend compositions as a function of FEF- loading are given in Figures (1- 5). It can be seen that EPDM pure sample loaded with different FEF content withstand stress more.

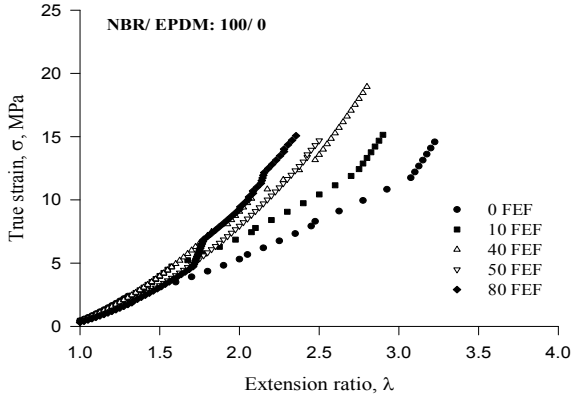

Fig. 1: Tensile stress- strain curves for FEF/ NBR composites

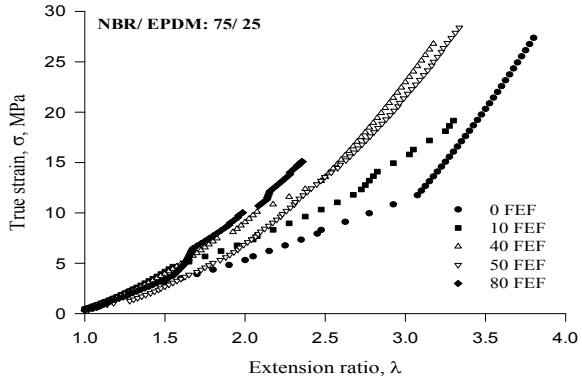

Fig. 2: Tensile stress- strain curves for NBR/ EPDM: 75/ 25 blend composites.

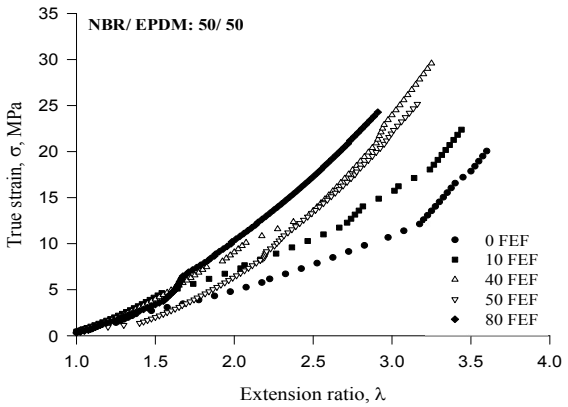

Fig. 3: Tensile stress- strain curves for NBR/ EPDM: 50/ 50 blend composites.

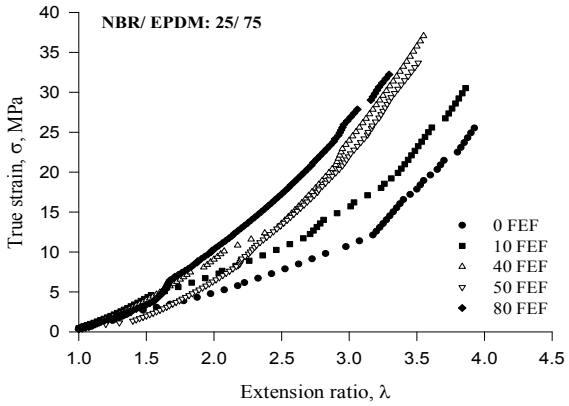

Fig. 4: Tensile stress- strain curves for NBR/ EPDM: 25/ 75 blend composites.

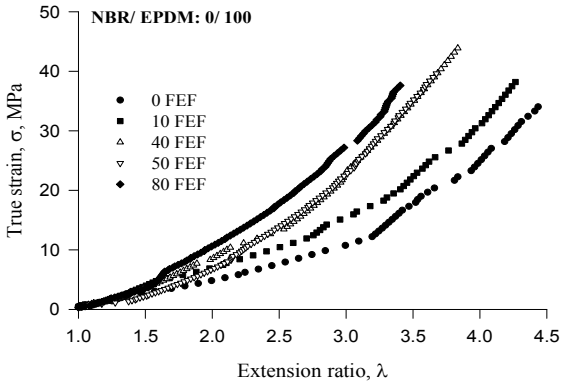

Fig. 5: Tensile stress- strain curves for FEF/ EPDM composites.

The variations in mechanical properties such as tensile strength and elongation at breaking point against filler loading for different blend composition are presented in Figures 6 and 7 respectively. It is clear that tensile strength of all blends increases with filler loading. However, the degree of reinforcement is found to be at its highest for pure EPDM rubber followed by 25/75 (NBR / EPDM) sample. The other

blend ratios showed relatively lower degree of reinforcement with FEF black. Both EPDM rubber and NBR are considered as non-self- reinforcing rubbers and FEF-black is regarded as being reinforcing filler [20]. Generally, non-self- reinforcing rubbers are reinforced through incorporation of reinforcing fillers.

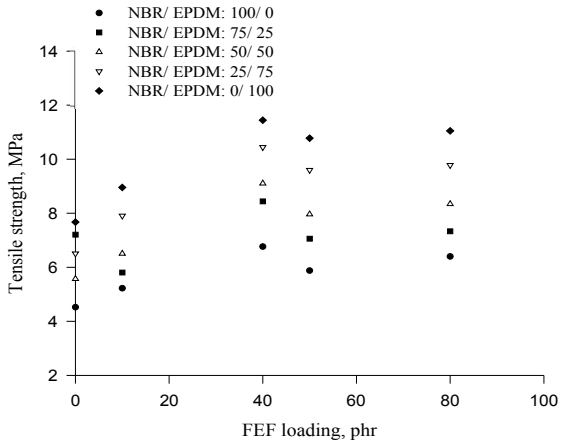

Fig. 6: The variation of tensile strength against filler loading for various blends.

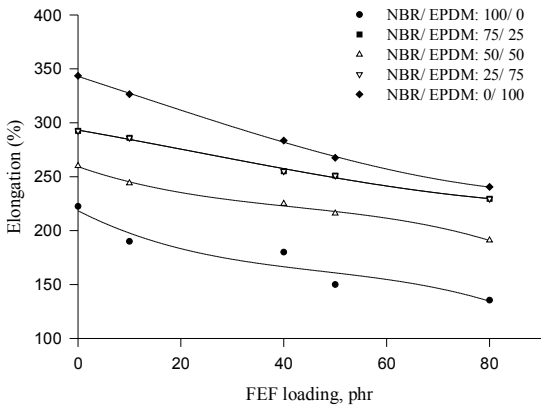

Fig. 7: Elongation at breaking point vs. filler loading for various blends.

The elongation at breaking point shows a decreasing behavior against filler loading for all systems. At very low filler loadings, when the matrix is not sufficiently reinforced, it can not sustain load and so failure occurs at lower elongations. By increasing filler loading, the molecular mobility decreases owing to the formation of physical bonds between filler particles and polymer chains (which is the basis for the mechanism of reinforcement). Consequently, the elongation at breaking point drops with increase in filler loading.

However, a higher elongation at breaking point is observed for EPDM and EPDM – rich blends, mainly because EPDM matrix has greater chain mobility than that of NBR matrix since the T_g of EPDM is -80^{0}C, whereas for NBR it is -40 ^{0}C [20].

3.2 Crosslinking density (η)

The crosslink density of an elastomer can be determined from swelling or mechanical measurements. The crosslinking density values of the homo polymers and blends have been calculated by using the following equation [21].

$$\eta = \frac{F}{2 A_0 \rho_p RT (\lambda - \frac{1}{\lambda^2})} \qquad (1)$$

Where F is the force required to stretch a specimen to an extension ratio (λ), A_0 the cross sectional area of the sample, R is the universal gas constant and T, the absolute temperature and ρ_p is the material density. The crosslinking density values are given in Table (3).

This high crosslinking density of 80 phr FEF loaded EPDM composite accounts for the superior properties of this composition. Meanwhile, one could observe that η increases with both CB content and EPDM concentrations.

Table (3): Crosslink density of NBR/ EPDM blend composites

Filler (phr)	Crosslinking density (η), mol/ kg			
	NBR/EPDM 0/ 100	NBR/EPDM 25/ 75	NBR/EPDM 50/ 50	NBR/EPDM 75/ 25
0	822	647	645	632
40	1285	1070	1106	764
80	1760	1340	1150	1066

3.3 Theoretical modeling

The mechanical properties of two-phase composites made up of a continuous polymer phase and particulate filler phase have been studied in great detail. The mechanical properties of particulate-filled composites are affected by a number of parameters such as filler orientation, filler/matrix adhesion and filler shape. In the literature, a number of theories and equations have been developed to predict the properties of the composites. The efficiency of load transfer from matrix to filler in a composite is strongly related to the optimum mechanical properties of the composite [22].

Following Johnson and Thomas [23] mechanical properties, we try to elucidate our results by modifying this treatment as follows. Theoretical treatment of the data of various composite models such as the parallel model, the series model, the Kerner model and the Kunori model were made use of to study the mechanical behavior of the blend. The parallel model (higher – upper – bound model) is given by the equation [24]

$$M = M_1 \phi_1 + M_2 \phi_2 \quad (2)$$

Where M is the mechanical property of the blend and M_1 and M_2 are the mechanical properties of the components 1 and 2, respectively and φ_1 and φ_2 are the volume fractions of the components 1 and 2 respectively. Here the components are considered to be arranged parallel to one another so that the applied stress elongates each of the components by the same amount.

In the lowest – lower –bound – series model, the components are arranged in series with the applied stress and are given by the equation [3]

$$\frac{1}{M} = \frac{\phi_1}{M_1} + \frac{\phi_1}{M_2} \quad (3)$$

Kunori et al [25] suggested a model when strong adhesive force exists between the blend components. In this model, the dispersed phase will contribute to the strength of the blend and the equation is:

$$\sigma_b = \sigma_m (1 - A_d) + \sigma_d A_d \quad (4)$$

Considering two possible fracture paths in a blend, Equation (4) can be modified as follows depending on whether the fracture is through the interface or through the matrix. When the fracture is through the interface:

$$\sigma_b = \sigma_m (1 - A_2^{2/3}) + \sigma_d \phi^{2/3} \quad (5)$$

when the fracture is through the matrix:

$$\sigma_b = \sigma_m (1 - \phi_d) + \sigma_d \phi_d \quad (6)$$

Where σ_b , σ_m and σ_d are the properties of the blend, matrix phase and dispersed phase respectively and φ_d is the volume fraction of the dispersed phase.

Figure (8) shows the theoretical and experimental curves of the tensile strength values of FEF/ EPDM composites. One may conclude that equation (5) (proposed by Kunori et al) gives a better fitting with the experimental data with respect to the other models.

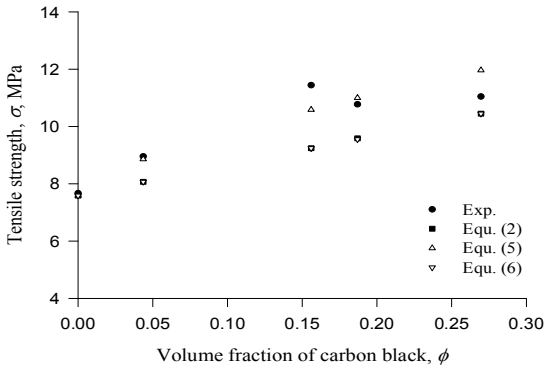

Fig. 8: Tensile strength vs. filler volume fraction fitted by difference models.

Conclusion

Results presented in this paper point to the existence of FEF carbon black loading, over which the properties of the NBR/ EPDM different blend ratio vulcanizates i.e., tensile strength and elongation at break are seen to pass through relatively sharp changes when plotted against the carbon black loading level. The presence of EPDM in the blend to accompanied by an enhancement in technological properties, which depends on the composition of both FEF and EPDM. The improvement in mechanical properties is supported by data on the increased content of crosslink density in these samples (obtained from stress – strain data measurements) upon FEF and EPDM loading. The degree of reinforcement achieved through incorporation of FEF – black is the highest for pure EPDM rubber followed by samples enriched with EPDM.

Kunori et al equation was found to give better fitting with the experimental results of the dependence of tensile strength on the FEF black loading.

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Reducing the Amount of Mineral Nitrogen Fertilizers for Red Globe Grapevines by Using Different Sources of Organic Fertilizers

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Abstract: This investigation was conducted for three successive seasons (2009, 2010 and 2011) in a private vineyard located at El-Khatatba, Menoufiya governorate; on mature Red Globe grapevines to study the possibility of reducing the amount of mineral nitrogen fertilizers for Red Globe grapevines by using different sources of organic fertilizers. The chosen vines were five years old, grown in a sandy loam soil, spaced at 2 X 3 meters apart, irrigated by the drip system, and cane-pruned and trellised by the Spanish Parron system. Ammonium sulphate 20.5% was added as a source of mineral fertilization. Organic fertilizers (compost and chicken manure) were applied either in the form of individual or mixed at 1:1 ratio on the basis of organic manure content of nitrogen. Thirteen treatments were applied as follows: 100%mineral nitrogen (control), 75%mineral nitrogen+ 25%compost, 75%mineral nitrogen + 25%chicken manure, 75% mineral nitrogen + 25%mixed manure, 50%mineral nitrogen + 50%compost, 50% mineral nitrogen + 50%chicken manure, 50% mineral nitrogen + 50%mixed manure, 25% mineral nitrogen + 75%compost, 25% mineral nitrogen + 75%chicken manure, 25%mineral nitrogen + 75%mixed manure, 0%mineral nitrogen + 100%compost, 0% mineral nitrogen + 100%chicken manure and 0% mineral nitrogen + 100%mixed manure. The results revealed the possibility of using organic nitrogen fertilizers as a partial substitute of mineral nitrogen fertilizers. This study indicated that application of 50%mixed manure included compost and chicken manure combined with application of 50%mineral nitrogen fertilizer was the best management system for achieving the best yield with its components as well as the best physical properties of bunches, improving the physical and chemical characteristics of berries, reducing nitrate and nitrite content of berries and ensuring the best vegetative growth parameters, leaf chlorophyll and leaf mineral content of Red Globe grapevines.
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Keywords: Grape, Red Globe, Mineral, Nitrogen, Fertilizer, Compost, and Chicken manure

1. Introduction

Fertilization is one of the most important cultural practices carried out during the growing season, especially nitrogen fertilization. Nitrogen is one of the major plant nutrients, being a part of protein, enzymes, amino acids, polypeptides and many other biochemical compounds in plant system i.e. encouraging cell division and the development of meristeniatic tissue (Nijjar, 1985 and Mengel and Kirkby, 1987).

One of the most important problems facing grape growers concerning the use of mineral nitrogen fertilizers is the high cost of the manufactured fertilizers needed for grapevines. Besides, the excess of mineral nitrogen fertilizers causes a major pollution of ground water with nitrate. Nitrogen fertilizer causes an accumulation of harmful residual substances, such as nitrate and nitrite in berries and leaves of grapevines (Ibraheem, 1994 and Montasser et al., 2003).

Organic fertilization is another option for supplying macro and micro nutrients necessary for plant growth (Harhash and Abdel-Nasser, 2000 and Elhaggar et al., 2004). In addition, the organic materials improve soil structure, aeration, and retention of moisture and reduce soil pH (Yagodin, 1984; EL-Nagar, 1996 and Nassar, 1998).

The possibility of using the organic fertilization for increasing growth and improving nutritional status of grapevines was approached by many researchers (Zhu and Zhu, 2000; Guo et al., 2000; Kassem and Marzouk, 2002; Hussein et al., 2005 and Omar, 2005).

The ultimate goal of this study was achieving the possibility of reducing the amount of mineral nitrogen fertilizers by using different sources of organic fertilizers (compost, chicken manure and mixed compost and chicken manure) for Red Globe grapevines.

2. Material and Methods

This investigation was conducted for three successive seasons (2009, 2010 and 2011) in a private vineyard located at El-Khatatba, Menoufiya governorate; on mature Red Globe grapevines to study the possibility of reducing the amount of mineral nitrogen fertilizers for Red Globe grapevines by using different sources of organic fertilizers. The chosen vines were five years old, grown in a sandy loam soil, spaced at 2 X 3 meters apart, irrigated by the drip system, and cane-pruned and trellised by the Spanish Parron system. The vines were pruned during the last week of December for the three seasons of the study so as to maintain a load of 72buds/vine (6canes X 12buds/vine). The vineyard was fertilized at the rate of 60 units nitrogen/Feddan.

Ammonium sulphate 20.5% as a source of mineral fertilization was added at three times: 25% was added at the beginning of bud burst, 50% after fruit set and 25% after harvest.

Organic fertilizers (compost and chicken manure) were applied either in the form of individual or Mixed Manure at 1:1 ratio on the basis of organic manure content of nitrogen. Organic fertilizers were applied once in the soil at the second week of January of each season.

The physical and chemical properties of the experimental soil, compost and chicken manure are shown in Table (1).

The normal horticultural practices were applied to all Red Globe vineyards, except those dealing with nitrogen fertilization treatments.

Two hundred and sixty uniform vines were chosen. Each five vines acted as a replicate and each four replicates were treated by one of the following treatments.

Thirteen treatments were applied as follows:

1) 100% Mineral Nitrogen (control)
2) 75% Mineral Nitrogen + 25% Compost
3) 75% Mineral Nitrogen + 25% Chicken Manure
4) 75% Mineral Nitrogen + 25% Mixed Manure
5) 50% Mineral Nitrogen + 50% Compost
6) 50% Mineral Nitrogen + 50% Chicken Manure
7) 50% Mineral Nitrogen + 50% Mixed Manure
8) 25% Mineral Nitrogen + 75% Compost
9) 25% Mineral Nitrogen + 75% Chicken Manure
10) 25% Mineral Nitrogen + 75% Mixed Manure
11) 0% Mineral Nitrogen + 100% Compost
12) 0% Mineral Nitrogen + 100% Chicken Manure
13) 0% Mineral Nitrogen + 100% Mixed Manure

The following parameters were adopted to evaluate the tested treatments:-

Representative random samples of 6 bunches/vine were harvested at maturity when TSS in berry juice reached about 16-17% according to Tourky *et al*., (1995). The following characteristics were determined:

1. Yield and physical characteristics of bunches:

Yield/vine (kg) was determined as number of bunches/vine X average bunch weight (g). Also, average bunch weight (g), bunch length and width (cm) were determined.

2. Physical characteristics of berries:

Average Berry weight (g), average berry size (cm^3) and average berry dimensions (length and diameter) (cm) were determined.

3. Chemical characteristics of berries:

Total soluble solids in berry juice (TSS%) were determined by a hand refractometer and total titratable acidity was calculated as tartaric acid (%) (A.O.A.C. 1985). Hence TSS /acid ratio and total anthocyanin of the berry skin (mg/100g fresh weight) according to Husia et al., (1965) were calculated. Nitrate (NO_3) and Nitrite (NO_2) were determined according to the method of Sen and Donaldson (1978).

4. Some characteristics of vegetative growth

1- Average shoot diameter (cm).
2- Average shoots length (cm).
3- Average number of leaves/shoot.
4- Average leaf area (cm^2) of the apical 5^{th} and 6^{th} leaves using a CI-203- Laser Area-meter made by CID, Inc., Vancouver, USA.
5- Total leaf area/vine (m^2) was determined by multiplying average number of leaves/shoot by average leaf area then by the number of shoots per vine.

5. Leaf total chlorophyll and mineral content

1-Leaf content of total chlorophyll was measured by using nondestructive Minolta chlorophyll meter SPAD 502 of the 5^{th} and the 6^{th} leaves (Wood *et al.,* 1992).
2-Leaf content of total nitrogen (%) (Pregl, 1945), phosphorus (%) (Snell and Snell 1967) and potassium (%) (Jackson, 1967) were determined.

- Statistical analysis:

The complete randomized block design was adopted for the experiment. The statistical analysis of the present data was carried out according to Snedecor and Chocran (1980). Averages were compared using the new L.S.D. values at 5% level.

3. Results and Discussion

1. Yield and physical characteristics of bunches:

Data in (Table, 2) show that the yield and its components of Red Globe grapevines were greatly affected by mineral and organic fertilizers in the three seasons of this study.

The highest value of yield was obtained with vines receiving 75% mineral nitrogen fertilizer + 25% organic fertilizer followed in a descending order by 100% mineral nitrogen fertilizer in the first season. However, in the second and third seasons, data revealed that vines receiving 50% mineral nitrogen fertilizer + 50% organic fertilizer recorded the highest values followed in a descending order by 75% mineral nitrogen fertilizer + 25% organic fertilizer then 100% mineral nitrogen fertilizer + 0% organic fertilizer. On the other hand, vines receiving 0% mineral nitrogen fertilizer + 100% organic fertilizer recorded the lowest values in the all seasons of the study.

With respect to number of bunches/vine, it is obvious that no significant differences could be detected among fertilizer treatments in the first season. While, in the second and third seasons, results showed that vines receiving 50% mineral nitrogen fertilizer + 50% organic fertilizer recorded the highest values followed in a descending order by 75% mineral nitrogen fertilizer + 25% organic fertilizer then 100% mineral nitrogen fertilizer + 0% organic fertilizer. On the other hand, vines receiving 0% mineral nitrogen fertilizer + 100% organic fertilizer recorded the lowest values in the three seasons of this study.

As for the effect of fertilizer treatments on physical characteristics of bunches i.e. (bunch weight, bunch length and bunch width), it was noticed that vines receiving 75% mineral nitrogen fertilizer + 25% organic fertilizer recorded the highest values for these parameters followed in a descending order by 100% mineral nitrogen fertilizer in the first season. However, in the second and third seasons, data revealed that vines receiving 50% mineral nitrogen fertilizer + 50% organic fertilizer recorded the highest values followed in a descending order by 75% mineral nitrogen fertilizer + 25% organic fertilizer then 100% mineral nitrogen fertilizer + 0% organic fertilizer. On the other hand, vines receiving 0% mineral nitrogen fertilizer + 100% organic fertilizer recorded the lowest values in the three seasons of this study.

As regards type of organic fertilizers, it is obvious that the mixed manure recorded the highest values, followed in a descending order by chicken manure, while compost recorded the lowest values in the three seasons of this study.

The beneficial effects of using organic fertilizers along with mineral nitrogen fertilizer on increasing yield and bunch weight & length could be due to their effect on providing vines with their requirements from different nutrients at a longer time as well as their effect on increasing the availability of nutrients in the soil for uptake by plants and enhancing the nutritional status of the vines in favour of yield and cluster weight (Nijjar, 1985).

Table (1): Physical and chemical properties of the soil, compost and chicken manure

	Soil	Compost	Chicken Manure
Sand (%)	**72.1**	**-**	**-**
Silt (%)	**2.6**	**-**	**-**
Clay (%)	**25.3**	**-**	**-**
Texture	**Sandy loam**	**-**	**-**
Weight of m^3 (kg)	**-**	**450**	**520**
Moisture content (%)	**-**	**21**	**24**
PH	**7.65**	**7.72**	**7.79**
Field capacity (%)	**19.5**	**-**	**-**
Ca Co_3 (%)	**0.7**	**-**	**-**
EC (Mmhos/cm)	**1.52**	**1.83**	**1.92**
Organic matter (%)	**1.7**	**47.3**	**58.9**
Organic carbon (%)	**0.79**	**27.3**	**28.5**
N (%)	**0.32**	**1.74**	**1.93**
C/N ratio	**2.47**	**15.69**	**14.77**
P (%)	**0.09**	**0.73**	**0.57**
K (%)	**0.52**	**0.96**	**0.82**

Table (2):Effect of organic and mineral nitrogen fertilizers on yield/vine and physical characteristics of bunches in Red Globe grapevines in 2009, 2010 and 2011 seasons

Characteristics	Yield/vine (kg)			Average number of bunches			Average bunch weight (g)			Average bunch length (cm)			Average bunch width (cm)		
Treatments	2009	2010	2011	2009	2010	2011	2009	2010	2011	2009	2010	2011	2009	2010	2011
100% Mineral Nitrogen (Control)	17.32	18.79	20.95	21.8	24.1	26.6	794.5	779.7	787.7	21.7	21.7	22.1	14.1	13.8	14.2
75% Mineral Nitrogen + 25% Compost	17.70	19.07	21.29	22.0	24.1	26.7	804.5	791.2	797.4	21.8	21.8	22.3	14.3	14.0	14.2
75% Mineral Nitrogen + 25% Chicken Manure	17.81	19.47	21.86	22.1	24.2	26.9	805.7	804.5	812.8	21.9	22.0	22.3	14.4	14.0	14.3
75% Mineral Nitrogen + 25% Mixed Manure	18.11	19.88	22.06	22.3	24.4	27.0	812.2	814.6	817.0	22.2	22.1	22.4	14.6	14.1	14.5
50% Mineral Nitrogen + 50% Compost	16.11	20.25	22.71	21.5	24.5	27.2	749.2	826.6	835.1	21.4	22.3	22.6	13.7	14.3	14.6
50% Mineral Nitrogen + 50% Chicken Manure	16.81	20.57	23.07	21.7	24.6	27.3	774.8	836.3	844.9	21.4	22.4	22.7	13.8	14.4	14.7
50% Mineral Nitrogen + 50% Mixed Manure	17.02	20.93	23.35	21.7	24.9	27.5	784.4	840.5	849.2	21.5	22.6	22.9	14.0	14.6	15.0
25% Mineral Nitrogen + 75% Compost	15.27	17.50	19.63	21.1	23.8	26.2	723.9	735.4	749.3	21.1	21.3	21.7	13.3	13.4	13.9
25% Mineral Nitrogen + 75% Chicken Manure	15.36	17.65	19.79	21.2	23.8	26.4	724.4	741.6	749.7	21.1	21.5	21.8	13.5	13.5	13.9
25% Mineral Nitrogen + 75% Mixed Manure	15.73	17.95	20.06	21.4	23.9	26.4	735.1	750.9	758.6	21.2	21.5	22.0	13.5	13.7	14.0
0% Mineral Nitrogen + 100% Compost	14.31	16.22	18.04	20.8	23.3	25.8	687.8	696.1	699.4	20.6	20.9	21.4	12.9	13.1	13.4
0% Mineral Nitrogen + 100% Chicken Manure	14.78	16.87	18.78	20.9	23.5	25.9	707.0	718.0	725.0	20.8	21.0	21.5	13.0	13.2	13.6
0% Mineral Nitrogen + 100% Mixed Manure	14.88	17.18	19.27	20.9	23.6	26.1	712.1	727.8	738.4	20.9	21.2	21.5	13.2	13.2	13.7
new L.S.D. at 0.05 =	0.43	0.35	0.27	N.S.	0.3	0.2	4.9	4.2	4.1	0.5	0.2	0.1	0.4	0.2	0.1

The results is this concern go in line with Darwish et al. (1996) on Roomy Red grapevines, Harhash and Abd El-Nasser (2000) on King Ruby grapevines, Abd EL-Hameed and Rabeea (2005) on Superior seedless grapevines and Belal (2006), who indicated that adding 60 units organic nitrogen from any source + 20 units mineral nitrogen/feddan gave the highest increase of cluster weight and yield/vine in Thompson seedless grapevines.

2. Physical characteristics of berries:

The results presented in (Table, 3) revealed that all physical characteristics of berries i.e. average berry weight, average berry size, average berry length and average berry diameter were significantly affected by the conducted treatments in the three seasons of the study. It was found that vines receiving 75% mineral nitrogen fertilizer + 25% organic fertilizer recorded the highest values of these parameters followed in a descending order by 100% mineral nitrogen fertilizer in the first season. However, in the second and third seasons, data revealed that vines receiving 50% mineral nitrogen fertilizer + 50% organic fertilizer recorded the highest values followed in a descending order by 75% mineral nitrogen fertilizer + 25% organic fertilizer then 100% mineral nitrogen fertilizer + 0% organic fertilizer. On the other hand, vines receiving 0% mineral nitrogen fertilizer + 100% organic fertilizer recorded the lowest values in the three seasons of this study.

Table (3): Effect of organic and mineral nitrogen fertilizers on physical characteristics of berries in Red Globe grapevines in 2009, 2010 and 2011 seasons

Characteristics / Treatments	Average berry weight (g)			Average berry size (cm^3)			Average berry length (cm)			Average berry diameter (cm)		
	2009	2010	2011	2009	2010	2011	2009	2010	2011	2009	2010	2011
100% Mineral Nitrogen (Control)	8.69	8.51	8.82	8.42	8.24	8.52	2.89	2.87	2.92	2.81	2.79	2.84
75% Mineral Nitrogen + 25% Compost	8.78	8.64	8.91	8.53	8.35	8.60	2.92	2.89	2.93	2.85	2.80	2.85
75% Mineral Nitrogen + 25% Chicken Manure	8.93	8.79	9.06	8.67	8.48	8.73	2.94	2.89	2.94	2.86	2.82	2.86
75% Mineral Nitrogen + 25% Mixed Manure	8.97	8.85	9.12	8.70	8.53	8.79	2.97	2.92	2.94	2.90	2.85	2.87
50% Mineral Nitrogen + 50% Compost	8.38	8.94	9.28	8.13	8.67	8.98	2.83	2.94	2.98	2.76	2.86	2.90
50% Mineral Nitrogen + 50% Chicken Manure	8.43	9.12	9.39	8.17	8.83	9.08	2.86	2.97	2.99	2.78	2.90	2.91
50% Mineral Nitrogen + 50% Mixed Manure	8.56	9.15	9.43	8.29	8.88	9.16	2.88	3.01	3.02	2.80	2.93	2.94
25% Mineral Nitrogen + 75% Compost	7.99	8.18	8.51	7.72	7.87	8.18	2.77	2.81	2.85	2.69	2.73	2.76
25% Mineral Nitrogen + 75% Chicken Manure	8.06	8.21	8.46	7.79	7.89	8.13	2.80	2.82	2.87	2.73	2.74	2.80
25% Mineral Nitrogen + 75% Mixed Manure	8.12	8.26	8.52	7.84	7.92	8.18	2.82	2.85	2.90	2.73	2.78	2.82
0% Mineral Nitrogen + 100% Compost	7.78	7.95	8.11	7.55	7.69	7.82	2.68	2.71	2.77	2.60	2.64	2.69
0% Mineral Nitrogen + 100% Chicken Manure	7.86	8.01	8.23	7.61	7.74	7.93	2.70	2.74	2.79	2.63	2.65	2.73
0% Mineral Nitrogen + 100% Mixed Manure	7.94	8.12	8.38	7.68	7.83	8.07	2.73	2.76	2.82	2.66	2.68	2.75
new L.S.D. at 0.05 =	0.05	0.03	0.04	0.1	0.05	0.06	0.05	0.04	0.03	0.05	0.03	0.02

Mixed manure as a source of organic fertilizers was recorded the best as compared to chicken manure and compost in the three seasons of this study.

The positive effect of organic fertilizer treatments on berry weight and size may be attributed to the increase of organic matter content and improvement of the structure and physical properties of the soil (Gamal, 1992).

These results are in agreement with those obtained by Ahmed et al., (2000) on Flame seedless grapevines, Harhash and Abd EL-Nasser (2000) on flame seedless and Belal (2006) who indicated that 60 units organic nitrogen + 20 units mineral nitrogen gave the highest values of cluster length, berry length and berry width as compared with the other treatments, especially in case of using mineral nitrogen alone.

3. Chemical characteristics of berries:

As shown in (Table, 4), it is apparent that all berry chemical properties i.e. total soluble solids, titratable acidity, TSS/acid ratio, anthocyanin and nitrate and nitrite content of berries were significantly affected by fertilizers treatments in all seasons of this study.

The highest values of total soluble solids, TSS/acid ratio and anthocyanin and the lowest values of acidity were obtained with vines receiving 75% mineral nitrogen fertilizer + 25% organic fertilizer followed by 100% mineral nitrogen fertilizer in the first season. However, in the second and third seasons, data revealed that vines receiving 50% mineral nitrogen fertilizer + 50% organic fertilizer recorded the highest values followed by 75% mineral nitrogen fertilizer + 25% organic fertilizer then 100% mineral nitrogen fertilizer + 0% organic fertilizer. On the other hand, vines receiving 0% mineral nitrogen fertilizer + 100% organic fertilizer recorded the lowest values of total soluble solids, TSS/acid ratio and anthocyanin and the highest values of acidity in the three season of this study.

Mixed manure as a source of organic fertilizers was recorded the best as compared to chicken manure and compost in the three seasons of this study.

Table (4): Effect of organic and mineral nitrogen fertilizers on chemical characteristics of berries in Red Globe grapevines in 2009, 2010 and 2011 seasons

Characteristics	TSS (%)			Acidity (%)			TSS/acid ratio			Total anthocyanin (mg/100g F.W.)			Nitrate (mg/kg F.W.)			Nitrite (mg/kg F.W.)		
Treatments	2009	2010	2011	2009	2010	2011	2009	2010	2011	2009	2010	2011	2009	2010	2011	2009	2010	2011
100% Mineral Nitrogen (Control)	16.3	16.4	16.5	0.58	0.58	0.56	28.1	28.3	29.5	24.3	24.5	25.7	17.88	18.37	18.56	1.83	1.87	1.89
75% Mineral Nitrogen + 25% Compost	16.4	16.4	16.6	0.58	0.58	0.55	28.3	28.3	30.2	24.6	24.6	26.3	14.65	15.40	15.61	1.46	1.52	1.54
75% Mineral Nitrogen + 25% Chicken Manure	16.6	16.5	16.7	0.57	0.57	0.54	29.1	28.9	30.9	25.3	25.1	27.6	12.88	13.37	13.56	1.40	1.44	1.45
75% Mineral Nitrogen + 25% Mixed Manure	16.7	16.6	16.7	0.56	0.56	0.53	29.8	29.6	31.5	25.9	25.7	27.7	10.58	10.97	11.02	1.32	1.36	1.39
50% Mineral Nitrogen + 50% Compost	16.1	16.6	16.8	0.61	0.55	0.53	26.4	30.2	31.7	22.6	26.3	28.0	9.86	10.19	10.32	0.79	0.73	0.82
50% Mineral Nitrogen + 50% Chicken Manure	16.2	16.7	17.0	0.60	0.55	0.52	27.0	30.4	32.7	23.1	26.5	28.8	9.60	9.70	9.90	0.74	0.69	0.77
50% Mineral Nitrogen + 50% Mixed Manure	16.3	16.8	17.1	0.59	0.54	0.51	27.6	31.1	33.5	23.7	27.4	29.7	9.08	9.27	9.37	0.71	0.64	0.73
25% Mineral Nitrogen + 75% Compost	15.9	16.1	16.4	0.63	0.61	0.58	25.2	26.4	28.3	21.3	22.6	24.4	8.52	8.72	8.82	0.25	0.23	0.27
25% Mineral Nitrogen + 75% Chicken Manure	16.0	16.2	16.5	0.62	0.61	0.57	25.8	26.6	28.9	22.0	22.7	25.1	7.80	8.10	8.50	0.22	0.19	0.25
25% Mineral Nitrogen + 75% Mixed Manure	16.1	16.3	16.5	0.61	0.60	0.56	26.4	27.2	29.5	22.5	23.3	25.6	7.17	7.28	7.42	0.17	0.15	0.18
0% Mineral Nitrogen + 100% Compost	15.7	15.9	16.2	0.67	0.64	0.60	23.4	24.8	27.0	20.3	21.1	22.9	5.31	5.43	5.51	0.09	0.07	0.10
0% Mineral Nitrogen + 100% Chicken Manure	15.8	16.0	16.3	0.65	0.63	0.59	24.3	25.4	27.6	20.4	21.6	23.8	4.71	4.79	4.83	0.08	0.07	0.09
0% Mineral Nitrogen + 100% Mixed Manure	15.8	16.1	16.3	0.64	0.62	0.58	24.7	26.0	28.1	21.0	22.3	24.2	4.47	4.52	4.54	0.06	0.04	0.07
new L.S.D. at 0.05 =	0.3	0.1	0.2	0.0	0.01	0.01	0.9	0.7	0.8	1.1	0.9	0.7	5.41	5.69	5.81	0.75	0.72	0.78

Table (5): Effect of organic and mineral nitrogen fertilizers on morphological characteristics of vegetative growth in Red Globe grapevines in 2009, 2010 and 2011 seasons

Characteristics	Average shoot diameter (cm)			Average shoot length (cm)			No. of leaves/shoot			Average leaf area (cm2)			Total leaf area/vine (m2)		
Treatments	2009	2010	2011	2009	2010	2011	2009	2010	2011	2009	2010	2011	2009	2010	2011
100% Mineral Nitrogen (Control)	1.03	1.04	1.09	177.1	177.3	183.5	28.3	27.3	28.9	187.7	187.2	189.9	26.6	25.5	27.4
75% Mineral Nitrogen + 25% Compost	1.05	1.05	1.11	178.4	179.8	185.9	28.9	28.0	29.5	189.0	189.7	192.3	27.3	26.5	28.4
75% Mineral Nitrogen + 25% Chicken Manure	1.09	1.05	1.13	180.7	181.3	187.3	29.1	28.7	30.1	191.6	191.2	193.7	27.8	27.5	29.2
75% Mineral Nitrogen + 25% Mixed Manure	1.13	1.07	1.14	181.8	183.8	189.7	29.8	29.3	30.6	192.8	193.6	196.0	28.7	28.4	30.0
50% Mineral Nitrogen + 50% Compost	0.99	1.11	1.17	171.2	185.3	191.1	27.0	29.5	30.8	181.0	194.8	197.1	24.4	28.7	30.3
50% Mineral Nitrogen + 50% Chicken Manure	0.99	1.13	1.18	173.5	187.8	193.5	27.0	30.5	31.6	183.6	197.3	199.5	24.8	30.1	31.5
50% Mineral Nitrogen + 50% Mixed Manure	1.02	1.16	1.22	174.8	189.1	194.7	27.6	31.3	32.3	185.2	198.4	200.5	25.6	31.1	32.4
25% Mineral Nitrogen + 75% Compost	0.96	1.01	1.05	163.1	170.5	175.4	25.1	26.1	27.7	174.4	179.3	182.0	21.9	23.4	25.2
25% Mineral Nitrogen + 75% Chicken Manure	0.97	1.01	1.06	166.6	172.3	178.7	25.3	26.7	28.3	176.0	182.4	185.3	22.2	24.4	26.2
25% Mineral Nitrogen + 75% Mixed Manure	0.98	1.02	1.08	165.4	173.0	177.8	25.9	27.3	28.9	176.8	181.6	184.2	22.9	24.8	26.6
0% Mineral Nitrogen + 100% Compost	0.93	0.97	1.02	155.4	160.0	167.7	23.5	24.8	25.9	163.7	171.4	174.1	19.3	21.2	22.6
0% Mineral Nitrogen + 100% Chicken Manure	0.94	0.99	1.03	156.8	164.3	170.5	24.1	25.4	26.8	168.2	174.4	175.7	20.3	22.2	23.6
0% Mineral Nitrogen + 100% Mixed Manure	0.96	0.99	1.05	159.7	168.7	172.0	24.7	25.9	27.2	172.5	175.8	178.5	21.3	22.8	24.3
new L.S.D. at 0.05 =	0.05	0.03	0.04	1.5	1.3	1.2	1.1	0.7	0.6	1.3	1.1	0.9	1.2	0.8	0.7

This result may be due to that organic fertilizers are rich in their content of macro and micro elements which led to enhance in photosynthesis, this means that more sugar

(glucose) is available for growth and fruit ripening (Keller et al., 1998).

These results are in accordance with Harhash and Abd EL-Nasser (2000) and Belal (2006) who found the gradual increasing of organic nitrogen doses to 60 units with decreasing the dose of mineral nitrogen to 20 units gave the highest significant increase of TSS %, TSS/acid ratio and lowest significant decrease of total acidity of Thompson seedless grapevines.

With respect to nitrate and nitrite content of berries, it is obvious that nitrate and nitrite content of berries were significantly decreased by reducing the amount of mineral nitrogen fertilizer. The highest values of nitrate and nitrite content of berries was obtained with vines receiving 100% mineral nitrogen fertilizer followed in a descending order by 75% mineral nitrogen fertilizer + 25% organic fertilizer. While, vines receiving 100%, 75% and 50% organic fertilizer recorded the lowest values in the three seasons of this study.

Concerning type of organic fertilizers, it is obvious that the mixed manure recorded the lowest values, followed in an ascending order by chicken manure, while compost recorded the highest values in the three seasons of this study.

This may be ascribed to that using organic fertilizers are often considered as a desirable nitrogen source because the nitrogen is in the mineralization immobilization cycle longer and thus is more slowly available (Hailberg and Keeney, 1993). Moreover, the use of organic manure (as slow release for nitrogen) induced a further reduction in N03-N accumulation in the plant compared with mineral nitrogen (as fast release for nitrogen) EL-Sisy (2000).

The obtained data are in agreement with those reported by Harhash and Abd EL-Nasser (2000) and Belal (2006), who reported that the interactions of all combinations between organic nitrogen plus mineral nitrogen doses gave a significant decrease in nitrate and nitrite content in the juice of berries as compared with mineral nitrogen alone for Thompson Seedless grapevines.

4. Some characteristics of vegetative growth

Results presented in (Table, 5) revealed that vegetative growth parameters (shoot diameter, shoot length, number of leaves per shoot, leaf area, and total leaf area/vine) were significantly affected by the applied fertilizers in the three seasons of the study. It was found that vines receiving 75% mineral nitrogen fertilizer + 25% organic fertilizer recorded the highest values of these parameters followed in a descending order by 100% mineral nitrogen fertilizer in the first season. However, in the second and third seasons, data revealed that vines receiving 50% mineral nitrogen fertilizer + 50% organic fertilizer recorded the highest values followed in a descending order by 75% mineral nitrogen fertilizer + 25% organic fertilizer then 100% mineral nitrogen fertilizer + 0% organic fertilizer. On the other hand, vines receiving 0% mineral nitrogen fertilizer + 100% organic fertilizer gave the lowest values in this respect.

Mixed manure as a source of organic fertilizers was recorded the best as compared to chicken manure and compost in the three seasons of this study.

The beneficial effect of organic fertilizers on leaf area of plants could be related to the improvement of physical conditions of the soil, providing energy from microorganisms activity, increasing nutrient supply and, improving the efficiency of macro elements as well as its ability to meet some micronutrient requirements (Cook, 1982; Tisdale et al., 1985; Kolble et el., 1995 and EL-Nagar, 1996).

The data go in line with those reported by Abou-Taleb (2004) on pecan trees and Belal (2006), who indicated that application of 60 units organic nitrogen + 20 units mineral nitrogen/feddan significantly increased leaf area as compared with the other applied treatments.

5. Leaf total chlorophyll and mineral content

As shown in (Table, 6), it is obvious that leaf content of total chlorophyll and percentages of total nitrogen, phosphorus and potassium were significantly affected by fertilizer treatments in the three seasons of this study.

With respect to leaf total chlorophyll content, it was found that vines receiving 75% mineral nitrogen fertilizer + 25% organic fertilizer recorded the highest values followed by 100% mineral nitrogen fertilizer in the first season. However, in the second and third seasons, data revealed that vines receiving 50% mineral nitrogen fertilizer + 50% organic fertilizer recorded the highest one followed by 75% mineral nitrogen fertilizer + 25% organic fertilizer then 100% mineral nitrogen fertilizer + 0% organic fertilizer. On the other hand, vines receiving 0% mineral nitrogen fertilizer + 100% organic fertilizer recorded the lowest values in the three seasons of this study.

As regards type of organic fertilizers, it is obvious that the mixed manure recorded the

highest values, followed in a descending order by chicken manure, while compost recorded the lowest values in the three seasons of this study.

The beneficial effect of organic fertilizers in increasing total chlorophyll may be due to more uptakes of nutrients such as nitrogen, magnesium and iron which are involved in chlorophyll formation as mentioned by Harhash and Abd EL-Nasser (2000).

The obtained results are in agreement with those reported by Belal (2006), who found that using different doses from organic manure and mineral N fertilizers significantly increased total chlorophyll in the leaves, the highest values were obtained from using 60 units of organic fertilizer + 20 units of mineral nitrogen per feddan.

Concerning the effect of fertilizers on leaf mineral content, it is apparent that vines receiving 75% mineral nitrogen fertilizer + 25% organic fertilizer were the most efficient in nitrogen, phosphorous and potassium uptake followed by 100% mineral nitrogen fertilizer in the first season. However, in the second and third seasons, data revealed that vines receiving 50% mineral nitrogen fertilizer + 50% organic fertilizer recorded the highest one followed by 75% mineral nitrogen fertilizer + 25% organic fertilizer then 100% mineral nitrogen fertilizer + 0% organic fertilizer. On the other hand, vines receiving 0% mineral nitrogen fertilizer + 100% organic fertilizer recorded the lowest values in the three seasons of this study.

Mixed manure as a source of organic fertilizers was recorded the best as compared to chicken manure and compost in the three seasons of this study.

The improving effect of organic fertilizers on leaf content of nitrogen, phosphorus and potassium can be attributed to their influence manifested in increasing the organic matter in the soil (Nijjar, 1985) which by its turn increases the soil water holding capacity, which improves the solubility and consequently the availability of nutrients (Zaid and Kriem, 1992; EL-Kassas et al., 1997 and Nasser, 1998). Also, the addition of organic manures leads to the production of humates which can be exchanged for adsorbed anions (Cook, 1982).

The results in this respect are in accordance with those of Darwish et al. (1996) on Roomy Red grapevines, Harhash and Abd EL-Nasser (2000) on Flame seedless grapevines, Abd EL-Hameed and Rabeea (2005) on Superior grapevines and Belal (2006) who mentioned that adding 60 units organic nitrogen from any source + 20 units mineral nitrogen/feddan gave the highest values of N, P and K content in the leaf petioles as compared with the other treatments used in Thompson seedless grapevines.

Table (6):Effect of organic and mineral nitrogen fertilizers on leaf total chlorophyll and mineral content in Red Globe grapevines in 2009, 2010 and 2011 seasons

Characteristics	Leaf total chlorophyll content (SPAD)			Leaf nitrogen content (%)			Leaf phosphorus content (%)			Leaf potassium content (%)		
Treatments	2009	2010	2011	2009	2010	2011	2009	2010	2011	2009	2010	2011
100% Mineral Nitrogen (Control)	37.61	37.92	38.82	2.08	2.08	2.19	0.39	0.37	0.39	1.60	1.61	1.66
75% Mineral Nitrogen + 25% Compost	38.30	38.40	39.20	2.12	2.09	2.22	0.40	0.38	0.40	1.63	1.63	1.67
75% Mineral Nitrogen + 25% Chicken Manure	38.80	38.80	39.50	2.14	2.11	2.25	0.41	0.38	0.41	1.65	1.65	1.68
75% Mineral Nitrogen + 25% Mixed Manure	39.50	38.85	39.65	2.17	2.14	2.27	0.43	0.40	0.42	1.68	1.65	1.69
50% Mineral Nitrogen + 50% Compost	36.37	40.10	40.90	1.98	2.15	2.28	0.37	0.41	0.43	1.55	1.71	1.74
50% Mineral Nitrogen + 50% Chicken Manure	36.70	40.40	41.30	1.98	2.19	2.31	0.37	0.43	0.44	1.56	1.72	1.76
50% Mineral Nitrogen + 50% Mixed Manure	37.40	41.20	41.90	2.03	2.21	2.34	0.38	0.44	0.46	1.59	1.75	1.79
25% Mineral Nitrogen + 75% Compost	34.80	36.60	37.30	1.90	1.98	2.10	0.33	0.35	0.38	1.47	1.54	1.57
25% Mineral Nitrogen + 75% Chicken Manure	35.13	37.09	38.09	1.91	2.03	2.14	0.34	0.36	0.38	1.48	1.56	1.61
25% Mineral Nitrogen + 75% Mixed Manure	36.00	37.60	38.10	1.94	2.05	2.17	0.35	0.36	0.39	1.53	1.60	1.62
0% Mineral Nitrogen + 100% Compost	33.70	34.26	36.06	1.80	1.91	1.99	0.30	0.33	0.35	1.42	1.45	1.52
0% Mineral Nitrogen + 100% Chicken Manure	34.19	35.13	35.83	1.83	1.92	2.03	0.32	0.34	0.37	1.44	1.48	1.51
0% Mineral Nitrogen + 100% Mixed Manure	34.51	36.06	36.96	1.87	1.96	2.06	0.33	0.34	0.37	1.46	1.52	1.56
new L.S.D. at 0.05 =	0.83	0.74	0.59	0.0	0.01	0.02	0.04	0.01	0.02	0.05	0.03	0.02

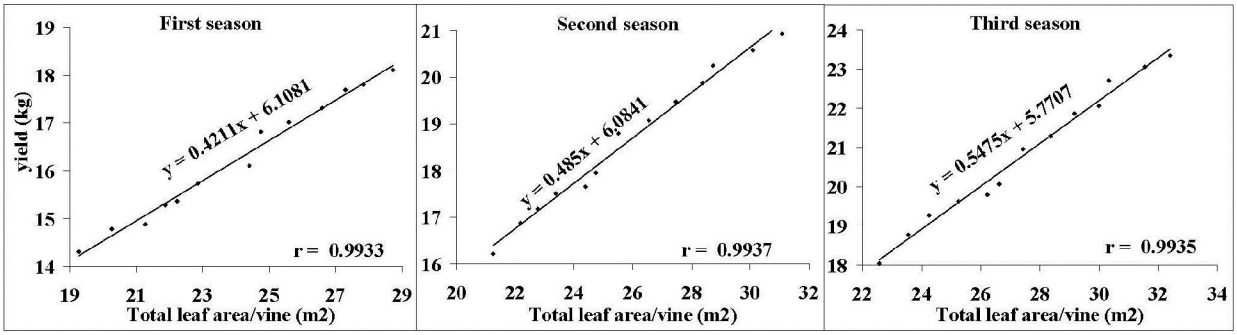

Fig (1): The relationship between the total leaf area (m^2) and yield (kg) in the three seasons

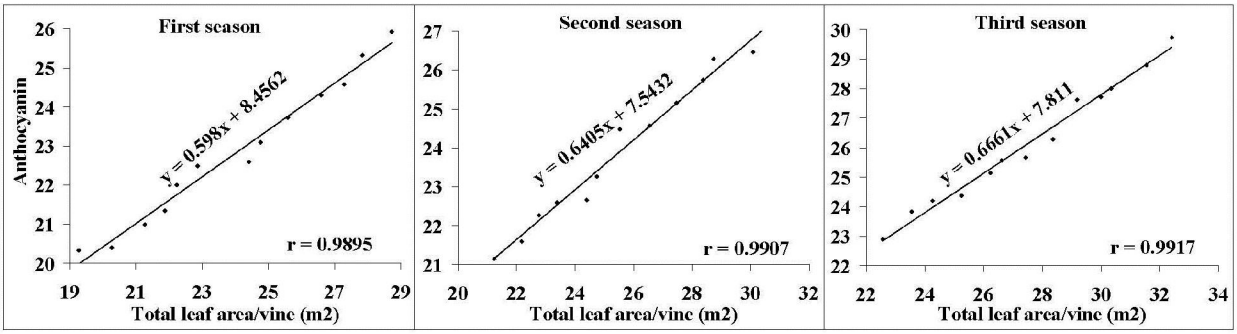

Fig (2): The relationship between the total leaf area (m^2) and anthocyanin (mg/100g F.W.) of berry skin in the three seasons

Data illustrated in Figures (1 & 2) indicated the existence of a highly positive correlation between total leaf area per vine (m^2) and yield (kg) and between total leaf area per vine (m^2) and anthocyanin content of berry skin (mg/100g F.W.) in the three seasons.

From the foregoing results, it can be concluded that there is a possibility of using organic nitrogen fertilizers as a partial substitute of mineral nitrogen fertilizer. However, this study confirmed that application of 50%Mixed Manure organic fertilizers included compost and chicken manure combined with application of 50% mineral nitrogen fertilizer was the best management system for achieving the best yield with its components as well as the best physical properties of bunches, improving the physical and chemical characteristics of berries, reducing nitrate and nitrite content of berries and ensuring the best vegetative growth parameters, leaf chlorophyll and mineral content of Red Globe grapevines.

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Power, Cooperation, Trust and Commitment in Supplier-Buyer Relationships

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Abstract: Facing rapid changes in technology and globalization of markets, firms become very difficult to live alone. The cooperation between buyer-supplier helps to improve the efficiency of the supply chain as a whole for the mutual benefits of both parties involved. Supplier's exercising power is a critical factor that influences cooperation. This study aims to investigate how supplier's use of power affects cooperation climate between a buyer and a supplier, buyer's trust in the supplier, and buyer's commitment from buyer's perspective. Their relationships are hypothesized and investigated based on the empirical data collected from companies in the Iran Cosmetics industry. The results demonstrate that use of non-coercive power improve cooperation and trust between the buyer and supplier. The buyer is inclined to make commitment to the supplier when cooperation and trust exist. Practical insights are provided accordingly in this paper to achieve closer buyer-supplier relationships in nowadays highly competitive environments.
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Keywords: exercised power, cooperation, trust, commitment

1. Introduction

In the competitive game of short time-to-market and differentiation to achieve customer satisfaction and loyalty, success for individual firms depends on how well the supply chain functions as a whole (Miles and Snow, 2007). A critical element achieving supply chain effectiveness is establishing cooperative relationship between buyers and suppliers (Skinner et al., 1992).

Interaction between two firms results in various contingencies, where the firms modify their resources to each others expectations. Power is the mechanism that can explain the relations of two firms (Emerson, 1962). Power is generally considered important for the understanding of buyer-supplier relationship (Frazier and Antia, 1995).

Cooperation refers to situations in which parties work together to achieve mutual goals, leading to outcomes that exceed what any of the firms involved in a supply chain would achieve if they acted solely in their own best interests (Anderson and Narus, 1990; Sahadev, 2005). It is defined as cooperation climate herein because it refers to situations. Cooperation requires two parties in a relationship to participate actively to achieve mutual benefits (Morgan and Hunt, 1994). Despite much discussion about the needs for cooperation, there are few studies considering power impacts on cooperative relationship. Most studies investigate power effects on performance of relationship, such as conflict (Brown et al., 1995; Lee, 2001; Benton and Maloni, 2005; Leonidou et al., 2008), satisfaction (Ramaseshan et al., 2006; Leonidou et al., 2008), commitment (Brown et al., 1995; Maloni & Benton, 1995, Benton & Maloni 2005; Ramaseshan et al., 2006), and trust. In this study, it is tried to find that how exercised powers influence cooperation climate, trust and commitment.

The purpose of this study is, therefore, to examine the roles of two types of exercised power as key driving forces in building buyer's commitment. The contribution of this study can be justifies on three major grounds: (a) it provides an explanation of the exercised power-cooperation-trust-commitment association; (b) it tires to shed light on the role of power sources in building buyer-supplier relationship, which, although critical, have received scant empirical attention within the semiconductor industry context; and (c) it concurrently tests the associations between a set of key behavioural constructs of the buyer-supplier relationship, using PLS.

2. Theoretical Background and Research Hypotheses

We reviewed the multi-disciplinary literature related to power, cooperation, trust and commitment and developed the conceptual framework shown in Figure 1. The framework consists of five basic constructs. The proposed conceptual linkage of these constructs is the following: exercised power (coercive and non-coercive) provides the starting points of the model and directly affects cooperation climate and trust, which act as mediating variables. In turn, these two constructs affects buyer's commitment.

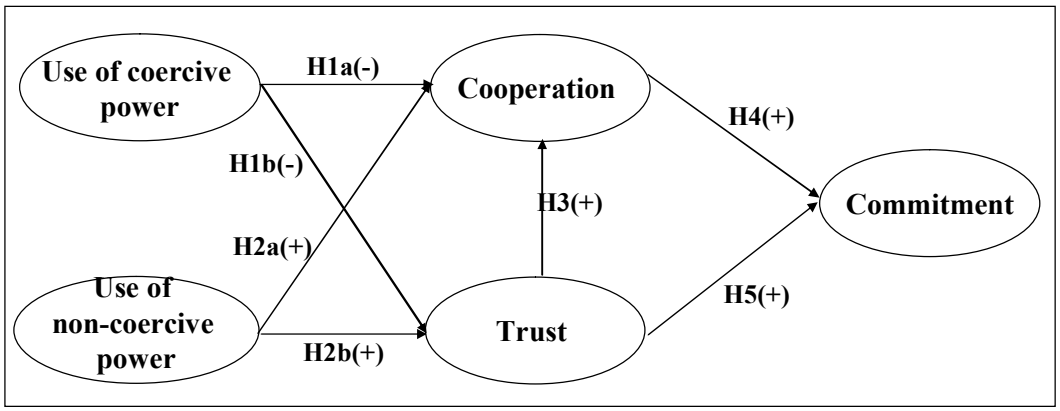

Figure 1. Research framework

2.1. Exercised Powers and Buyer-Supplier Cooperation Climate

Bases of power define the resources the supplier has available to influence buyer's decision in this study. These resources establish the foundation for the sentiments that characterize the behaviour process (Skinner et al., 1992).

Coercive power is based on the perception of one party in a relationship that the other has the ability to mediate punishment (El-Ansary and Stern, 1972). The exercise of this source of power reflects aggressive, forceful and suppressive behaviours, which essentially forces the other party in a relationship to do things that otherwise she or he would not have done. The use of coercive power is most likely to escalate tension and frustration in the relationship, because the one party performs actions of which the other disapprove, does not have the resources to carry out or feels offended by.

Cooperation climate is defined as a situation where similar or complementary coordinated actions are taken by firms in interdependent relationship to achieve mutual outcomes in this study. Exercising coercive power leads to the other party feels threatened. The other party may comply with the firm temporally. However, it will depress a firm's willingness to cooperation for a long-term because the firm does not feel the benevolence of the firm uses its coercive power (Dapiran and Hogarth-Scott, 2003; Maloni and Benton, 2000). Inevitably, this situation will result in disagreements between the two parties and will elevate conflicts to a manifest state. In turn, coercive power decreases the level of cooperation in a relationship. Dapiran and Hogarth-Scott (2003) contend that use of coercive power only lead to capitulation and will raise a desire, for the other party, to exit the relationship.

Non-coercive power can be derived from four basic sources: (1) reward, based on the perception of one party that the other has the ability to mediate reward; (2) legitimate, based on the perception of one party that the other has a legitimate right to prescribe behaviour; (3) referent, based on one party's identification with the other, and (4) expert, based on the perception of one party that the other has some special knowledge or expertise (Skinner et al., 1992). Exercising non-coercive power is a signal to demonstrate benevolence. Once other parties perceive the benevolence, it will invest more efforts in the relationship because when a firm demonstrates willingness to establish a benevolent relationship with the other firm, the other firm is under pressure to give much back to the firm. A cooperation situation will be achieved (Dapiran & Hogarth-Scott, 2003; Leonidou et al., 2008). Based on foregoing discussion, the following hypotheses are suggested.

Hypothesis 1a: Supplier's use of coercive power is negatively related to buyer-supplier cooperation climate.

Hypothesis.1b: Supplier's use of non-coercive power is positively related to buyer-supplier cooperation climate.

2.2. Exercised Powers and Trust

Trust has been defined as an expectation or willingness that exists between two parties (Sahadev, 2005). For example, it is defined as "a willingness to rely on an exchange partner in whom one has confidence" in Moorman, Deshpande and Zaltman (1992). The definitions tend to highlight a party's belief that the other party to the exchange is capable of being relied on in case of any need.

From the interpersonal relationship theory, analogies comparing the development of buyer-supplier relationships to loving relationships and marriage have been advanced for nearly two decades (Dwyer et al., 1987). Similar to a marriage relationship, perdition of how others firm will behave in certain situation determines one's behaviours. When a supplier uses non-coercive power, such providing

incentives or sharing critical information or resource to the other party in a help manner or favourable actions, it shows benevolence and reliability to the buyer. It of course suggests a positive linkage between non-coercive power and trust. Useful information and resources are quite important for product development and manufacturing to improve time-to-market, especially in cosmetics industry. Leonidou et al. (2008) find that use of non-coercive power decrease conflict and lower disunity between two parties and therefore prompts trust between two parties.

However, when a firm exercises its coercive power, the exercised power erodes the trust between interacting parties, mainly because such aggressive behaviour will prevent the formation of a belief that the partner is dependable, honest and fair (Dwyer et al., 1987; Leonidou et al., 2008). A benevolent partner will subordinate immediate self-interest for long range group gain and not take unexpected actions that would have a negative impact on the other firms. Therefore, use of coercive power, such as threaten and punishment, hurts trust (Anderson and Narus 1990). In light of the above reasoning, the following hypotheses are proposed.

Hypothesis 2a: Supplier's use of coercive power is negatively related to buyer's trust in supplier.

Hypothesis 2b: Supplier's use of non-coercive power is positively related to buyer's trust in supplier.

2.3. Trust and Cooperation Climate

Trust refers the belief that a party's word is reliable and a party will fulfil its obligation in an exchange (Mohr and Spekman, 1994). As an extension of personal relationship and negotiation theory, trust has been consistently mentioned as a predictor or an antecedent of cooperative behaviour between organizations (Johnston et al., 2004). When the trading partner is more reliable, dependable and benevolent so that its behaviours can be predictable, a firm will tend to invest more in cooperative behaviours and a cooperation situation will be achieved. Exchange relationship featuring trust will be able to manage greater stress and will display greater adaptability. Once trust is established, firms learn that joint efforts will lead to outcomes that exceed what the firm would achieve (Anderson and Narus, 1990). Mohr and Spekman (1994) suggest that the lack of trust will be deleterious to information exchange, to reciprocity of influence, and will diminish the effectiveness of joint problem solving.

We therefore posit a positive causal path from trust to cooperation climate. This path is supported by several researches such as Brunard and Kleiner (1994). In addition, within the context of a trusting relationship, firm sometimes are willing to postpone temporarily the receipt of their own outcomes until some later time (Anderson and Narus, 1990). In sum, we propose the following hypothesis.

Hypothesis 3: Buyer's trust in supplier is positively related to buyer-supplier cooperation climate.

2.4. Cooperation Climate and Commitment

Relationships evolve through four phases identified as (1) awareness, (2) exploration, (3) expansion and (4) commitment four phase (Dwyer et al., 1987). Commitment is the most advanced phase. Commitment refers to the willingness of partners to exert effort on behalf of the relationship. It suggests a future orientation in which partners attempt to build a relationship (Mohr and Spekman, 1994). A high level of commitment provides the context in which both parties can achieve individual and joint goals without raising the spectre of opportunistic behaviour. Because more committed partners will exert effort and balanced short-term problems with long-term goal achievement, higher levels of commitment are expected to be associated with partnership success (Leonidou et al., 2008).

Transaction Cost Theory suggests that commitment can be viewed as an investment in transaction-specific asset, which are difficult or impossible to redeploy when a relationship is terminated. Buyer will invest in commitment to reciprocate supplier's contribution in cooperation. For firms in cosmetics industry, when buyers will receive more technology supports and R&D knowledge from suppliers, they then will be reciprocated by greater access to market information for developing product and manufacturing technology. When both parties receive valued contribution from each other, each partner has strong motivation to build, maintain, strengthen and deepen the relationship (Prahinski and Benton, 2004). That makes it more likely that both buyer and supplier perceive their relationship as a win-win opportunity (Kumar et al., 1995). Therefore, cooperation will enhance buyer's desire to develop a stable relationship. Specifically, we hypothesize:

Hypothesis 4: Buyer-supplier cooperation climate between a buyer and a supplier is positively related to buyer's commitment in buyer-supplier relationship.

2.5. Trust and Commitment

Several researches suggest that the future of buyer-supplier relationships depends on the commitment made by the partners. In commitment relationship, short-term sacrifices are normally necessary to realize long-term benefits (Dwyer et al., 1987). Because commitment involves potential vulnerability and sacrifice, parties will seek only trustworthy partners and firms are unlikely to be committed unless trust is already established. Trustful

partners refrain from their opportunistic behaviors, so trust can reduce the risk to make commitment from the perspective of transaction cost theory. Also, when a partner won't take unexpected behaviors, the other party will have higher motivation to deepen their relationship. In accordance with the theory of transaction cost theory and interpersonal relationship theory, it is considered the trust as a precursor of commitment (Morgan and Hunt, 1994), so the following hypothesis is posited.

Hypothesis 5: Buyer's trust in supplier is positively related to buyer's commitment.

3. Research Methodology

3.1 Sampling and Data Collection

Data were collected via a questionnaire sent to companies in the cosmetics industry in Iran. The target informants were the managers or staffs having experiences in supply chain management or supplier interaction. These target populations were mostly in the department of purchasing, R&D, production planning and control, and supply chain management according to the practices in the cosmetics industry. The target populations were then asked to select a relationship in which the buying company had most contact frequencies with its suppliers.

The population included 261 cream package design firms, 13 shampoo production firms, 34 Beauty raw material providers, 4 quality control companies, and few companies providing other kinds of materials in Iran. An informant was selected from a company. 207 informants agreed to receive the questionnaires and questionnaires were received after several follow-up calls. The usable data used in this study were completed by 124 informants. The response rate was 57.14% based on the number of questionnaires distributed.

3.2 Questionnaire Design

This study includes five constructs, and they are use of coercive power, use of non- coercive power, cooperation climate, trust climate and buyer's commitment. Scale items to measure supplier's use of coercive power, use of non- coercive power were adopted from Leonidou et al., (2008). Relationship climate constructs of cooperation climate, trust and commitment were all adopted from Maloni and Benton (2000). Trust refers to one party's confidence in honesty and integrity of the other partner (Anderson and Narus, 1990). The measurement items are designed to measure buyer's perception of honesty and integrity of the supplier, that is, buyer's trust on the supplier. Commitment refers to the feeling of being emotionally impelled to maintain a long-term relationship (Benton and Maloni, 2005). Being same as the constructs of cooperation and trust, commitment is measured by capturing buyer's willingness to develop long-term relationship with its supplier.

4. Analysis Results

The partial least squares method is used to analyze the research framework. Industry of focal companies and the number of employees are used as control variables to control the variances derived from different industries and firm's scale.

4.1. Convergent and Discriminant Validity

Although all the constructs have been assessed for their unidimensionality in Section 3.2, this does not guarantee an acceptable measurement model (Lee, 2001). Convergent and discriminant validity are tested accordingly.

In PLS, alternative ways of judging multiple-item consistency are used. The methods look at (1) the reliability of the individual items that make up the measures (2) composite reliability of the items as a group and (3) the average variance extracted form the manufactured by each of the items (Fornell and Larker, 1981). Table 1 shows the summary of convergent validity checks. Individual item reliability is assessed using the item's loading on the production. 0.7 is the suggested minimum level for items loadings (Fornell and Larker, 1981). Composite reliability assesses the inter-item consistency, which should have a minimum value of 0.7. All of the scales demonstrated acceptable stability on this basis. The third standard for reliability is that the average variance extracted (AVE) from the production by the items should exceed 0.5 so that the items share at least half of their variance with the production. All scales performed acceptably on this standard. Therefore, the measures in this study demonstrated adequate support for convergent validity.

4.2. Hypotheses Testing

Path coefficients of the structural model are illustrated in Figure 2. All of paths were significant except the path between use of coercive power and trust.

Given the loadings in Figure 1, H1a was supported (=-0.252, t =-2.42), that is, supplier's use of coercive power hurts cooperation climate between buyer-supplier relationships. However, H1b was not supported (=-0.135, t =-1.59). Although the relationship direction is consistent to our prediction in the hypothesis, the use of coercive power had no significant effect on trust.

H2a (=0.372, t =3.92) and H2b (=0.466, t=2.37) were both supported. Use of non-coercive power will promote cooperation climate and trust

between buyer and supplier. In the contrary to using coercive power, it is a positive way to exercise power. It is noted that the impact of use of non-coercive power on trust is higher than that on cooperation climate and trust has significant effects on cooperation. It can be inferred that trust partially mediates the effects of exercised non-coercive power on cooperation climate. H3 was also supported in this model (=0.354, t =3.76). Exercised power explained considerable amount variance of cooperation climate and trust with R^2=0.339 and 0.278, respectively.

Given the loadings between commitment and its predictors, cooperation climate and trust, it is found that both two constructs have positive and significant effects on buyer's commitment. The analysis results supported H4 (=0.455, t =3.48) and H5 (=0.430, t=2.33). Higher level of cooperation climate and trust within a relationship lead to buyer's higher commitment and cooperation climate had higher effect. Cooperation climate has higher loading on buyer's assessment of commitment than does trust. Two predictors provide an R^2 of 0.242 for commitment. Overall, the conceptual model gained considerable support from the data.

Table 1. Convergent validity checks of constructs

Latent variables	Loadings	Composite Reliability	AVE
Use of coercive power			
UCP1	0.746	0.84	0.664
UCP2	0.922		
UCP3	0.903		
Use of non- coercive power			
UNP1	0.871	0.92	0.580
UNP2	0.915		
UNP3	0.862		
Cooperation climate			
CC1	0.749	0.88	0.563
CC2	0.853		
CC3	0. 743		
Trust			
TR1	0.894	0.86	0.676
TR2	0.876		
TR3	0.862		
Commitment			
CM1	0.885	0.91	0.579
CM2	0.829		
CM3	0.933		

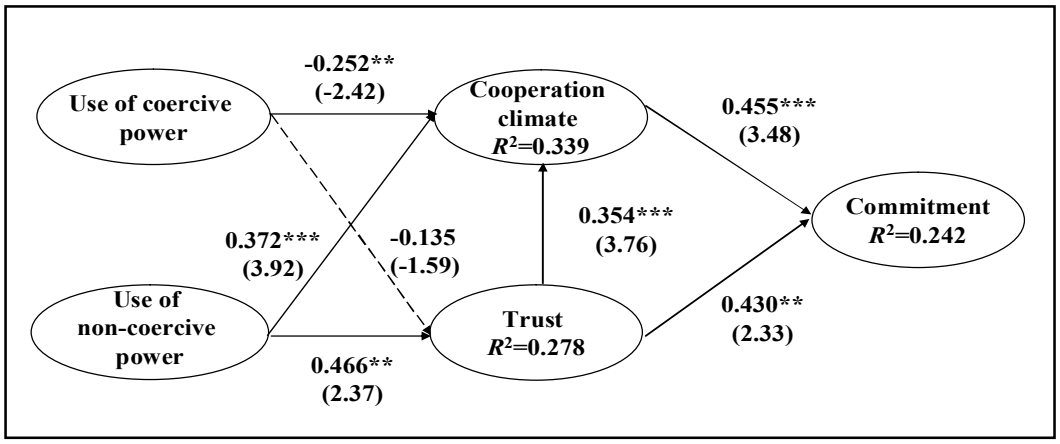

Figure 2. Empirical model with path loadings (significant paths are presented as solid lines)

5. Discussion and Conclusions

The associations among exercised power, cooperation climate, trust and commitment are studied in this study. Assuming cooperation and trust are the desired climate in buyer-supplier relationship, this study suggests that exercise non-coercive power has positive and significant effects on cooperation and trust. This usage of power should be used whenever possible. When using non-coercive power, both the cooperation climate level and trust will be improved. The results are similar to the finding in Benton and Maloni (2005), Maloni and Benton (2000), Dapiran and Hogarth-Scott (2003) and Skinner et al. (1992).

On the contrary, excised coercive bases of power were found to decrease the level of cooperation climate in the relationship. It is noted that use of coercive power has no significant influence on trust. We proposed several reasons in cosmetics manufacturing context. Respondents reported that the level of coercive power usage is not as high as that of the non-coercive power usage. The low usage frequency might not sufficient to catch respondents' attention or the extant of punishment

might not severe enough to erode trust between buyer and supplier. Usually, suppliers give punishment for contract issues with legitimacy, so the buyer won't think punishment is unreasonable. When the power is exercise under justice, it will be acceptable for the other side.

In the proposed model, trust influences cooperation and commitment positively and significantly. Trust leads to cooperation. Firms would not undertake cooperation without a sufficient level of trust initially (Johnston et al., 2004) and higher trust will results in higher cooperation. This result is also supported by Brunard and Kleiner (1994) and Kumar (1996).

Trust leads to commitment as well. The result is consistent with Leonidou et al. (2008) and trust-commitment theory (Morgan and Hunt, 1994). Frequent face-to-face contact, sharing of vital and proprietary information, exchanges of personnel and exposure to opportunistic behaviors, which derive trust, will help to increase buyer's commitment in staying and continuously investing in the relationship.

Cooperation climate is also positively related to commitment. When supplier invests more in cooperation, buyer will reward the supplier in accordance to basic concept of social excahnge theory. Commitment is a typical form of reward to retain a buyer-supplier relationship. Cooperation helps buyer and supplier to achieve win-win situation, as mentioned in Kumar et al. (1995).

In the future, strategies to increase cooperation between suppliers and buyers will grow in important. This study provides some initial guidance toward more cooperative relationships. Several strategies can be inferred from the testing results. From the testing result, it is found that the degree and source of power should be carefully used by the supplier in buyer-supplier relationships, because its direct and indirect effects on atmosphere constructs can lead to either harmonious or problematic results. Exercised coercive power reduces cooperation but exercised non-coercive power increases both cooperation climate and trust in the relationships. Only non-coercive powers are conductive to healthy buyer-supplier relationships.

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Multiagent Architecture for Management of Milk Tankers in Dairy Industry

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Abstract: In the business sector there has been and always will be a demand for a single master mind or an expert which can look after the crucial business activities round the clock throughout a year. This is not possible for any single human being as the business activities are either controlled by more than one human in the form of a team or by a single human in a discontinuous manner. This introduces an inconsistency in the decision making process. There has always been search for artificial methods to perform the same task in a consistent manner. This research paper has explored the way how intelligent multiagent system can be used to look after such activities in real time. In this paper the architecture of MAS for the management of milk tankers for a milk processing company is discussed. It involves a combination of knowledge base deduction methods with multi agent techniques, working together to accomplish this task. The financial aspects of the project are not included in the scope of this research. [Aslam Muhammad, Ahmad Zargham, Martinez-Enriquez A. M., Multiagent Architecture for Management of Milk Tankers in Dairy Industry. Journal of American Science 2011;7(8):827-832] (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Artificial Intelligence; Multiagent system.

1. Introduction

The software agents have been introduced just a couple of decades ago and ever since they have become so popular that public entities as well as private companies have spent a considerable amount of effort, time and investment in researches related to development of software agents. A software agent is embedded in a specified environment in which it is capable of achieving certain tasks with some degree of autonomy, i.e. without constant human guidance or intervention [1].

The next step in the field of software agents was the introduction of Multiagent systems (MAS). A multi-agent system (MAS) consists of multiple software agents that can interact with each other as well as with the environment in which they are placed. MAS can be used to solve problems that are difficult for an individual agent. MAS have an automated approach which has made human life very easy. MAS have proven that they are capable of solving real world problems, examples of applications are: coordinated defense system, transportation, industrial supporting robots, logistics, air traffic control and so on. Communication among agents is the key feature in MAS and agents work as a team, collaborating with other, this is done by means of passing messages to one another [2].

This research reports how MAS can be utilized in the management of milk tankers carrying milk for processing in a dairy industry. Right allocation of milk volumes at the right time for processing in any dairy industry is the vital key for optimizing the company business. Usually large dairy companies have more than one processing plants in a country or region and collect milk from a wide area cross the region. Allocation of milk to different processing units is the most important step of milk processing, from the collection of milk to it transportation to the factory where it is processed and after processing the milk products are dispatched to the market.

Pakistan dairy sector was selected as a case study model for the implementation of this multiagent system. Agriculture is the largest sector of the Pakistani economy, contributing 23% to the GDP of the country and involving 42% of the total labor force. Livestock is the largest of the various agriculture sub sectors. Milk is the largest commodity from the livestock sector accounting for 51% of the total value of the sector. Pakistan is the Fifth largest producer of milk in the world with a total production of 28 billion liter of milk a year, from a total herd size of 27 million milk animals (buffaloes and cows). There is a consensus among the stakeholders and the development experts that Pakistan's Dairy Sector has immense potential for growth [3].

Many factors affect the allocation of volumes of milk to different operational units including the forecast of milk volumes expected to be received in the regions, the processing capacity of different units, any maintenance activity in progress in any area of the processing unit, the type of product having higher demand in a particular season etc. Milk distribution is predominately an art in which knowledge, experience, engineering and supply chain demand all play important roles. Often there is more than one option available which are technically and

economically viable. In most of the present dairy industries milk allocation is made by experts on the basis of their extensive knowledge, past experience, and frequently 'intuition'. The development in the field of artificial intelligence and computer-based techniques in the recent years provide a way by which this process can at least be partially de-skilled.

This research paper aims at utilizing MAS to make decision in such a way that the outcomes of the decision making process are rational, consistent and correct. This is achieved through a combination of multi-agent system with a knowledge based system. Section 2 discusses the characteristics of the environment and agents involved in this multiagent architecture. It argues about the internal structure of individual intelligent agents. Section 3 shows how the multiagent architecture can be instrumented for real-world milk tanker management problems in a computationally and economically efficient way. Finally, in Section 4, the potentials and drawbacks of this system are discussed in order to come up with a set of conclusions respecting the applicability of multiagent technology to the milk distribution domain.

2. Related Work

Different related researches are present in following section:

2.1 Multiagent meta-model for strategic decision support

In this research the authors introduced a meta-model which generates multiagent system for strategic decision support. The system utilizes the new concepts of bargaining with learning to determine the cooperation between agents. Three essential functions form the original framework of the meta-model, provided with a technique of learning. System includes the search for typical plan of decisions while adapting to the problem and the search for the coalitions in the spatial and temporal dimensions. Overall the system allows an adaptation to all the types of strategic decision support [8].

2.2 Simulation and evaluation of urban bus-networks using a multiagent approach

The improvement in the service quality of public road demands analysis and planning tools. This need is mainly a result of evolution of the public road transportation systems. The existing range of road transportation simulation tools includes a variety of planning, training and demonstration tools. Out of these tools only a few cater the traveler behavior and public transportation specific vehicle operation. In this research, the authors introduce a bus-network simulation tools which include the above specificities. This toll also allows analyzing and evaluating a bus-network at diverse space and time scales. A multiagent approach is used to describe the global system operation as behaviors of numerous autonomous entities such as buses and travelers [9].

2.3 Milk-sense: a volatile sensing system recognizes spoilage bacteria and yeasts in milk

This research shows how an electronic nose unit containing 14 conducting polymer sensors can be used to detect the volatile profiles produced by uninoculated skimmed milk media or that inoculated with bacteria or yeasts when grown for time at some predefined temperature. It uses a discriminate function analyses (DFA) to separate unspoiled milk and that containing spoilage bacteria or yeasts. In this way the sensor array used behaves as a useful discriminator of microbial volatile profiles. This showed that it is possible to recognize, and differentiate, between species, the butanol and milk medium. Cross validation was made by using labeled individual replicates of treatments as unknowns demonstrated that it was possible to differentiate between (a) butanol controls; (b) unspoiled milk medium; (c) S. aureus; (d) K. lactis; (e) C. psuedotropicalis; and (f) B. cereus. The potential for using an electronic nose system for early detection of microbial spoilage of milk-based products is most certainly the method of future for detecting early milk spoilage [10].

3. Environment and agents in the system

The agents interact with the environment and as well as with the other agents present in the same environment. The architecture of the agents working in the environment is directly related with the complexity of the environment in which they operate.

3.1 Environment

The environment for the operation of agents in this study is inaccessible, in-deterministic, highly dynamic and continuous.

First of all inaccessible in the sense that the agent cannot possibly obtain complete up-to date information of the state of the environment because it is changing every moment. However complete up-to date information on the state of other agents is possible.

Any action which the agent takes will affect the environment, but up-to which extent it affects the environment and the achievement of goal for which the action the taken is not sure making it an in-determinant environment.

Like other situations the agent has partial control on the environment. For example the milk tankers were dispatched from the milk collecting centers and at the time of dispatch the collection center had calculated that it will reach the factory in 4 hours but it started to rain and the tankers got late. Form the collection center agent's point of view it did the right thing by postulating the time of arrival of tanker at the factory and communicating the factory agent

about the arrival time. But in reality the schedule couldn't be met. Such kind of scenarios such as raining, road blocks etc. makes the environment dynamic.

The rate at which the environment is changing is relatively slow. Like the when it starts to rain the system agents have sufficient time to cater the raining affects and plan course of action well in time so that the system design purpose is met.

The environment carries a large numbers of percepts and the actions which can be taken against them are also numerous. To store these percepts and actions a large amount of storage data is required for each gent in the system which will exponentially raise the cost of the system. To make the system economical the environment is treated as a continuous environment and agents are designed accordingly to cope with this environment to fulfill its goal

3.2 System agents

The agents working in the current environment include; milk tanker agent, milk collection agent, factory agent and business support agent. In addition to these agents an expert system also works in collaboration with these agents which determines the milk quality index in the tanker. Some of these agents do not have a 'learning by experience' module in them because the agents with reactive capability will serve the purpose well. In this way the learning module is not included in agent's architect and makes them simpler and fewer resources are required to realize these agents. The following section contains their functional and architectural details.

3.2.1 Milk tanker agent

The agent for a milk tanker is the simplest agent of the system. A functional or relational agent design serves the purpose of this agent completely. The tasks assigned to milk tanker agents include monitoring of physical and chemical properties of milk which include temperature, pH and acidity. After computing these parameters the agent communicates them to milk quality expert system. The expert system sets the tanker in priority in reception at the factory. The milk tanker agent also analyzes the rout path set by the tanker driver and estimates the time to reach the factory and communicates this time to factory agent so that it may know the time of arrival of milk tanker in factory. If the route changes due to rain or road blockage the driver updates the new route and again the milk tanker agent computes the time to reach the factory according to the new route and communicates again to the factory agent. Each taker is associated with a separate milk tanker agent having unique identity. This helps in establishing an effective communication channel among the agents.

3.2.2 Milk collection center agent

The milk collected at the milk collection centers across the country/regions. The milk collection center agent computes the total amount of milk received and its quality. Then accordingly distributes the amount of milk in tankers and allocates them to different factories. Besides from communication with milk tanker agents the milk collection agent also communicates with business support agent which gives instructions regarding the allocation of milk to different factories. It also gets updates of the status of factories from the business support agent.

3.2.3 Factory agents

This is the agent representing the recipient processing unit i.e. the factory. A factory contains different plants and each plant may contain more than one processing line. In this system an agent is associated with each line. This agent sends the conditions of the line such as any breakdown or maintenance plans to the plant agent. It also depicts the health and operational status of the line. After receiving this information from the line agents the plant agent analyze the data and sends a report to the factory agent about the condition down the line. In this way the plant agent in a way tells the factory agent that how much milk can be processed on each plant. The factory agent after analyzing the health of the system communicates with the other agent present outside the factory and bargains on the quantity of milk it requires to process. The hierarchy of the factory gents is shown in the fig below.

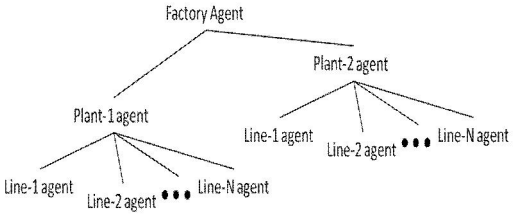

Figure 1. Hierarchy of the agents working inside a factory

2.2.4 Business support agent

This agent is like the supervisor of all the agents in the system. Its main responsibility, as clear from the name, is to support the company business. It controls the flow of information in the agent communication network and also has the additional role of resolving conflict among the agent in the system. It is fed with information such as the demand of different milk products in the markets and their sales trends in throughout the year. Using this information the agent postulates what product has the more priority and which should be made. Since it is also aware of the processing line types in different factories so it aides the milk collection center in distributing the milk to different factories. In case of any major breakdown on the line in any factory it

reacts instantly and generates a new distribution plan to milk collection center agents.

3.3 Milk quality expert system

The condition of milk in a tanker is the most important parameter to determine the priority of processing of the milk volume. In addition different dairy products require different quality profiles. Some products require higher pH while others require lower pH value. Similarly fat and non-solid fats ratio is also different for different products. The milk tanker agents communicate the physical and chemical parameters of the milk within the tanker. Using these parameters milk quality profiles are computed by the milk quality. These profiles are matched with the product profiles and the best match is selected. Also this agent computes the rate at which the quality degrades within the tanker. Because the milk acts as a buffer solution but if due to action of micro bacteria the milk loses its buffer nature and spoils rapidly within a few hours. So it is very important to predict the quality and process it before anything goes wrong. Milk pH plays an important role in this regard.

Microbial milk spoilage can severely affect the quality of the finish product and hence the commercial success of dairy products due to undesirable off-odors, physical defects and secondary metabolite toxicity. Important spoilage species include some Gram-negative bacteria such as Pseudomonas uorescens, Pseudomonas fragi and some Gram-positive species such as Bacillus cereus and Staphylococcus aureus [5]. Additionally, acidic conditions in milk fermentation products such as yoghurt and sour milk may favor the growth of some yeasts which can also generate undesired off-odors, and loss of texture quality. By monitoring the microbial load these effects can be minimized and this is the job of milk quality expert system.

4. Proposed System Architecture

The knowledge required by the multiagent system to make decisions regarding the management of milk tankers includes the condition of milk in the tanker, the operational capacity of different units, seasonality impacts, maintenance and breakdown situations, market sales forecasts and business priorities. These inputs act as parameters for the system and using them concludes a decision. In this study the individual agents working in the environment gather some of these parameters through perceptions but any action cannot be taken until the information not shared among the agents. Even if the information is shared between the agents an effective action is not possible if the agents do not cooperate with one another.

In multiagent systems it is an important and interesting endeavor to study the development of cooperative behavior among agents. Through cooperation among different functional agents it is possible to achieve a task which would be impossible to accomplish with only one agent. [6].

The MAS used in this research utilizes the decentralized coordination model for communication i.e. there is no master supervisor or coordinator in the system. The agents communicate with one another and by means of barging reach a decision. The communication network of the agents is shown in the fig. 2.

The process starts at the milk collection center where milk is received from the field. Now the milk collection agent computes the total amount of milk stock and using the information sent by the business support agent distributes the milk to different factories. The business support agent has the information such as the seasonality impacts, sale forecasts, the situation updates in different factories. In Pakistan there are mostly three season of milk production which are the Flush Season: (Jan to April) during this season there is maximum production of milk in the country due to rain and availability of fodder. Second is the lean Season (April to July) minimum production of milk in the country due to high environmental temperature, less green fodder availability and natural reproduction cycle of animals. Last is the semi-flush season (Aug to Dec) in this season about 70-80 % milk production takes place in the country [7].

After the distribution plan has been generated by the milk collection agent it communicates it to different milk tanker agents about it. Each milk tanker agent is given a unique identity through which it is recognized in the communication channel. The tankers are filled with milk and dispatched to different factories. During their journey to the destined factory, they keep monitoring the physical and chemical properties of the milk in the tanker through various sensors and communicate these parameters to milk quality expert system.

The milk quality expert system determines the quality profiles of the milk within the milk tanker and updates the factory agent about it so that the factory agent can prepare for its receiving for the suitable product. The milk quality expert system also determine if there is a chance that if milk is going to spoil so that it's receiving priority is revised and is given higher priority.

Once the milk tanker has reached the factory it waits for its turn to deliver the milk to the factory. The factory agent then informs the respective plant agent about the arrival of the stock. Then the respective plant starts processing the milk. In case if any sudden breakdown occurs in the factory the factory agent instantly communicates with the

business support agent and the other factory agents in the region for assistance. Now the bargaining starts within the factory agents under the super vision of the business support agent. If the situation does not get under controlled then the business support agent interferes in the bargaining process and imposes a decision on its own keeping the business objectives set earlier.

5. Case Study

An interesting scenario is presented in fig 3 below. The factory 1 receives milk from zones 1,2,3,4 and 5. This is shown by the green circle. Similarly for the factory 2 the crimson color circle covers partially zones 7 & 8 and complete zones 9 & 10. The zones 5 & 6 and some portions of zones 7& 8 are the intermediate zones and are in conflict between the two factories.

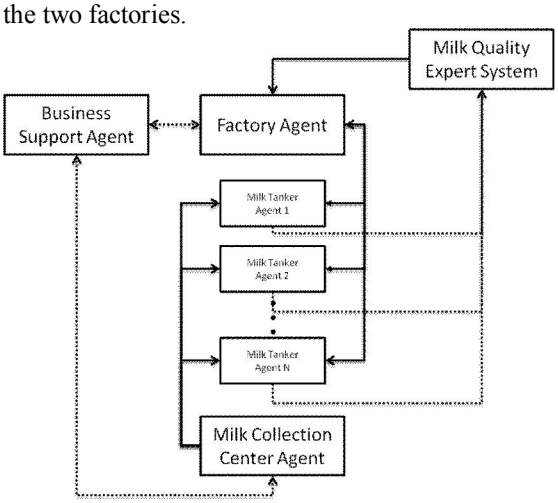

Figure2. The multiagent system architecture for the management of milk tankers in the dairy industry. The arrow heads indicate the direction of flow of information among different agents of the system.

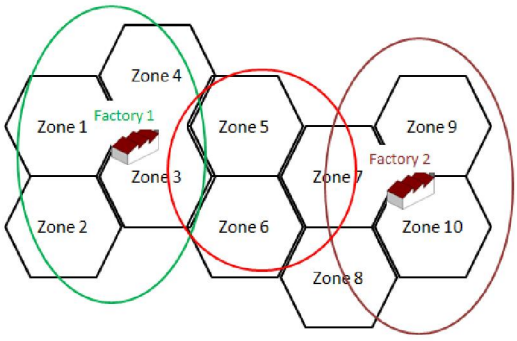

Figure 3. The green circle covers the zones covered by factory 1 while the crimson circle shows the zones covered by factory 3. The region encircled by the red intermediate circle indicates how conflict arises in these zones for allocation to both factories.

This conflict is resolved by the business support agent. This is achieved by using the facts and rules fed into the business support agent knowledge base. As discussed above the business support agent kames a decision keeping in view the business priorities, seasonality impacts, maintenance activities in progress in any factory and so on. In this way the milk collected from these zones goes to factory 1 and sometimes goes to factory 2. The system performance is monitored by the human user from time to time and there is the provision in the system to make small corrections. This is made possible by the fact that the same language of reasoning is used by both the system agents and human users. In this way it is possible for the user to calibrate the system from time to time. However most of the time the system runs autonomously yielding what would be termed as rational results. The overall performance of such a system adds valuable inputs to the company business.

6. Conclusion and Future Work

This paper presented a multiagent system approach for the management of milk tanker for a dairy industry. This work shows that the MAS techniques have an explicit effect on the existing conventional system. From the user's point of view, this system may be seen as an intelligent assistant capable to take useful decisions to support the business activity. This MAS mostly relies on the fact that the agents in the system apply reasoning procedures and use the bargaining to achieve the design objectives. The reasoning and bargaining of agents resemble those of the human operators making decision in the real world. In addition, the possibility for the business officials to access the knowledge bases and the ability to express this knowledge in understandable terms for the users make it feasible and easier to maintain and improve the knowledge base with their own experience and previous outcomes of the system when and where considered necessary. The use of a multi-agent system for routing of milk tankers benefits from the fact that with the passage of time the system gains experience and adds the newly gain knowledge in the existing data base for future decision making.

The beauty of MAS lies in the fact that it can incorporate any number of agents in its community. The greater the numbers of muti-functional agents the more complex problems are easily solved by it. In this research there is an opportunity for a route planner agent to assist the milk tanker driver (human user) in planning the shortest and economical path for the milk tanker transportation. After the milk tanker has been dispatched from the milk collection center a lot of factors can affect the itinerary of the milk

tanker e.g. raining, road blockage or any breakdown in the tanker itself. Any route planner agent can be utilized for this purpose however it will require additional communication protocols in order to meet this system design objectives. The incursion of new agent, however, will affect the overall system performance which will require a complete impact analysis before actually engaging the agent in action.

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Intelligent Assistant to Help Blind people for Selecting Wearable Items

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Abstract: We introduce intelligent assistant for the blind people that support them in selecting wearable items. Many intelligent systems have been developed for the people with impaired vision. These systems help them to deal with daily life problems like avoiding obstacles, face recognition, reading, and writing. However, blind persons still have to face a lot of problems in choosing their daily commodities .Therefore a system is needed for such people that can assist them in selecting their accessories. Our proposed system that is using text to speech technology for direct communication is intelligent enough to facilitate blind people in choosing their wearable items like dresses, shoes, hair styles, jewelry, and glasses according to the causal or formal events. The main purpose of this assistant is to make blind persons more independent and more confident so that they can enjoy life like healthy persons. The system's objectives are achieved with the unification of artificial intelligence particularly knowledge based system and image processing. We validate our system on various blind persons and get satisfactory results. [**Farzana Jabeen, Aslam Muhammad, Maliha Saleem Bakhshi, Martinez-Enriquez A. M**. Intelligent Assistant to Help Blind people for Selecting Wearable Items. Journal of American Science 2011;7(8):833-840] (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: vision impaired, text to speech technology, Blind assistance

1. Introduction

According to the world health organization surveys, the number of vision impaired people is estimated to be between 40 and 45 million [4]. In every era vision impaired people have to face a lot of problems in daily life that sighted people take for granted. In particular, these people face huge difficulties in reading, writing, moving freely in ever changing environments, and selecting their daily wearable items. As a result, blind people are in danger while moving on their own, and their sovereignty is limited. They have to depend upon on some of the friends or relatives to perform their daily life activities.

Above all huge estimated figure of vision impaired persons and their issues regarding daily life cannot be neglected. Many intelligent systems have been introduced to meet the needs of vision impaired people that help them in face recognition, reading, writing, avoiding obstacles. White cane [6] (purely mechanical device) is the most commonly used travel aid by the vision impaired people to avoid obstacles, uneven surfaces, holes, steeps and other hazards. Due to limited proximity detection (3-6 feet) and detection of obstacles with contact urged the scientists to introduce new mobile robots assistants (Navbelt, Guide cane) for better travel assistance of blinds. Robotic walker [8] is a more reliable device that is especially designed for indoor assistance of vision impaired persons. Grava architecture [7] is introduced with minimum modeling language for facial recognition.

Although all the existing intelligent systems are doing well at their own still blind persons have to face a lot of problems in choosing their accessories. A common belief exists that vision impaired people are not interested in their physical appearance. A sighted person can say this easily because he is independent and unaware about the difficulties that a vision impaired person has to face in the selection of daily commodities. Vision impaired person can make difference among the fabrics of their dresses but can never in case of colors, fashion, new trends. Some blind people organize their room wardrobe in a way that help them to select their dresses, shoes, ties, and pants etc. easily. For example they hang causal outfits on one side of the closet, and the formal on the other. When purchasing the new clothes, the blind people can distinguish only on the basis of fabrics and material of their accessories that may result in collection of similar colures. That's why blind people always needed the help of a sighted person so that he can assist them in shopping.

Unfortunately in our society it has become a norm that people are treated according to their way of dressing. So, blind people are hesitant to attend the formal functions because of the deficiency of selecting wearable items. This deficiency makes them realize their dependence on others. Vision impaired people want to become independent in their lives.

In this paper we have tried to tackle above issue.

Therefore, we propose an intelligent assistant agent system that assists blind people in selecting their wearable items like dresses, shoes, hair styles, jewelry, and glasses according to the causal or formal events. The main purpose of this assistant is to make blind persons more independent and more confident so that they can actively participate in every event.

The system objectives are achieved with the unification of artificial intelligence techniques (knowledge based system), digital image and signal processing (feature extraction, speech to text and text to speech conversion).The system gets input from sensors (gait detector, camera) and verbal description like (height, waist, and category) in order to facilitate the selection of wearable items. The system makes decision about the wearable items by using knowledge based system and then provides verbal description to the user regarding his getup. This system plays the role of human assistant, who helps the users in selecting wearable items.

Related work is presented in Section 2. Section 3 describes proposed system. Section 4 describes case study, in Section 5 conclusions and some future perspectives are given.

2. Related Work

Many intelligent agent systems have been developed for the assistance of blind persons that help them in increasing their limited sovereignty. This global system combines intelligent systems, virtual reality systems and multimodal interfaces in a house with domotics devices. The main purpose of this application is to support a blind person [3]. To complete system's objective multimodal interfaces, autonomous intelligent agents, virtual reality, and UML methodology are used. The system focuses on the facial expression to communicate with the blind users. This intelligent systems consists of two parts (a) a virtual character (the butler) who accomplishes all the tasks of butler. The butler interacts with the house and users, receiving perceptions and taking actions. (b) The system is a multi agent system. Each agent manages a specific module of the application and receives a set of perceptions that helps evaluate the environment and provides a set of actions.

Intelligent tutoring system (ITS) is designed to run on a handheld device with the aim to increase the independence of people with cognitive disabilities in industry and daily life activities [2]. ITS collects the idea of an active distributed support system where the knowledge of how to perform an activity is shared between people and artifact. This system consists of a Task Management System that includes a Content Management System and modules for ergonomic characteristics and personal information of users.

This work presents a review of existing locomotion assistance devices for the blinds. These devices measure the distance to the nearest obstacles and convey this information to the vision impaired people [1]. The proposed system introduces different methods for the distance measurement like infrared sensors, ultrasonic sensors, laser telemeters. This work also describes about the shortcomings of existing system and the methods to improve those systems.

This implicit interface is developed to assist the sighted persons so that they can easily choose entities like dress, shoes and hair style [5]. The proposed system is based on implicit Human Computer Interaction concept and knowledge based systems. System gathers customer information to facilitate the selection of daily life commodities.

A color recognition system is built on the smart phones for the blind people [10]. The user needs smart phone with a camera and to install this application on their phones. The proposed system has user interface with text to speech technology that helps in identifying object's colors.

All above intelligent systems assist the blind people in walking freely, face recognition, talk independently, color recognition and express their emotions properly in their discomforts. Still an intelligent system is missed that can help vision impaired persons in selection of their daily commodities.

3 Intelligent Talk-able Assistant for Blind (ITAB)

Our proposed system assists visual impaired people in shopping and selecting their daily life accessories according to their own taste. Text to speech technology with English language [9, 10, and 11] is used that not only makes system's interface friendly but also provides an efficient way of direct communication between user and system. Our system consists of two parts. Hardware support and software support. The hardware support shows the details of all physical devices (Figure 1) that help user to interact with the system and provides its personal and functional information according to the need of the software system. System inner working is explained with the help of Figure 2 that provides a broad vision about the role of agents.

The Figure 1 shows the physical components that describe about the hardware detail of ITAB. We are suggesting a trail room that is specifically dedicated for blind. The specialty of trial room is that all physical components of ITAB are set up there so that blind can easily interact with the system in isolation. Isolation helps user to describe about his/her desiring accessories openly in absence of any disturbance and human assistant.

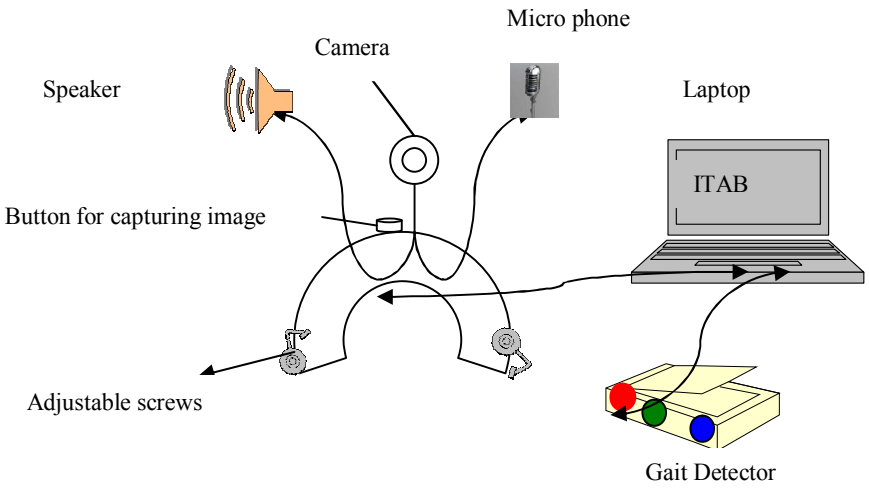

Figure 1: Model of Intelligent Talk-able Assistant for Blind (ITAB)

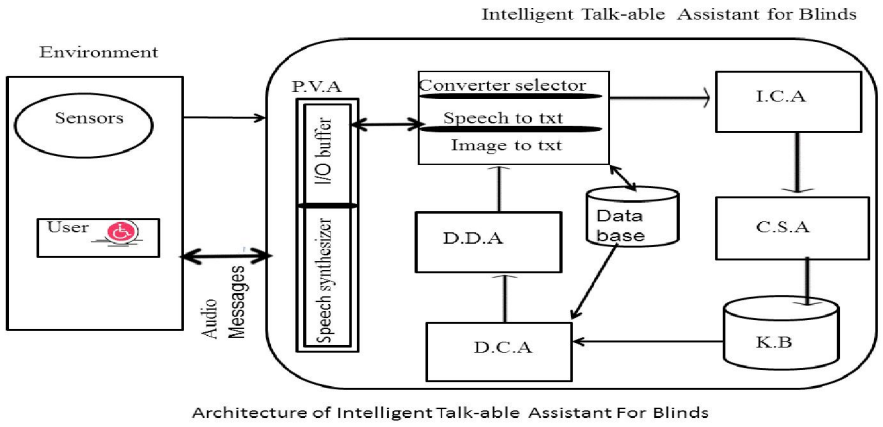

Figure 2 : Architecture of Intelligent Talk-able Assistant for Blind

Room front right corner is reserved with a camera that is adjusted to the top of the wall. It captures images from 3-D environment automatically and on user's demand by pressing the button on clutch. Speaker and microphone are fitted on the top left corner of the room. One ear speaker and micro phone that are connected with clutch is an optional way of communication between system and user.

ITAB consists of software components that act as intelligent agents to accomplish the tasks. Figure 2 shows the inner detail about the system's working and its components interaction. The system consists of following major components.

3.1 Perception vector agent (PVA)

ITAB uses sensors to get input from environment. (1) A movable camera is mounted on the top of the trial room's right corner as well as on the clutch that captures 3-D images continuously or on user's demand respectively. Camera sends captured images to the laptop that contains the ITAB software that help to get the exact skin tone color by using sampling mechanism [13] and facial

appearance of the user that is further used for selection of suitable wearable items. (2) Gait detector is a sensor that used to detect the walking style of the user [5]. This device consists of pressure sensors which are laid on some metallic surface. The user walks on this device bare footed and the pressure sensor detects the pressure of human foot from different angles and provides information like size, flat footed .This information is then sent to the ITAB system so that system can select appropriate shoes for the user. The walking device is provided by different lights: green color when nobody is walking on the device, blue when someone is walking on it and the system is getting information without any error, and red color when someone is walking and the system is not getting the correct input. A voice message is sent through speaker saying "Walk again".

User communicates with system either by using the trial room's speaker and microphone or one ear speaker and microphone connected with clutch. Instructions are provided by the ITAB to user with the speaker that guides the user how to interact with the system and what type of information system expects from user. System asks question regarding personal information, type of category and function.

User provides its personal (gender, height, weight, waist, and favorite color) and category selection information (dress, shoes, hair style, jewelry, glasses) relevant to event (causal, formal, sports, party) by using microphone.

Input from all sensors (image, data) and users (voice) are received by the perception vector that acts as an interactive agent with other agents of ITAB and user also. It consists of two components (a) I/O Buffers is a temporary storage for all input perception (voice, image, sensory input) and as well as output also. It contains input and output buffer that is used for the storage of input data from environment sensors and user and output data from convertor selector respectively (b) Speech synthesizer is a Text to speech system [10] with English language that converts the text into speech by producing artificial human voice. Speech synthesizer has its own database that is used to store all text description. By combining the pieces of text from the database Speech synthesizer is delivered audio messages to the user that can be listened by using speaker.

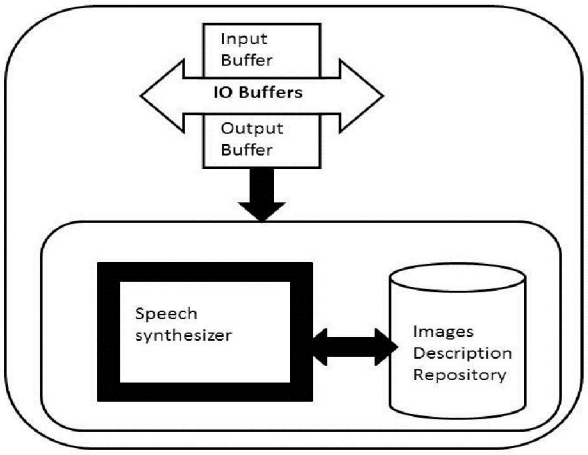

Figure: 3 Perception vector agent

Figure 3 : Perception Vector Agent

3.2 Converter Selection (CS)

PVA sends the perception input from I/O buffer to the Converter selector. First it saves a copy of user's image into database so that it can use that image for further processing during the next phases. Then Converter selector checks the format of the input whether it's an image or speech. Then on the basis of the input format it forwards data to the speech to text converter and image to text converter respectively. CSA uses existing models and algorithm for input conversion according to the format. Image to text converter uses the hamming trace transform method to extract the user's facial features [12].

The hamming trace transform method is combination of concept of Trace transform, Fourier transform and circus function to detect the facial feature from the target image. Skin tone is measured using the sampling mechanism [13] based on object detection by using machine learning approach.

AdaBoost algorithm is used for machine learning process. All the voice messages from the user are entertained by the speech to text converter that is used SPOC text to speech system [14].This system is used hidden Markov models for speech recognition that converts the speech into text.

3.3 Information Collector Agent (ICA)

All the text information from images and voice messages is sent back to the CSA that further transfers this data to the information collector agent. ICA extracts its desiring information like, gender, sex, height, weight, waist, skin color tone, favorite color, flat footed, foot size, high sole, and event nature from the data and forwards the information to the Category selector agent.

3.4 Category selector agent (CSA)

CSA manages different wearable items categories like dress, shoes, jewelry, and glasses on the basis of events nature, size, and seasonal stuff. Events can be wedding, party, sports day, convocation etc. Size can be small, medium, large and extra-large that depends upon height of the user. Seasonal stuff is categorized according to winter, summer and spring. CSA selects the wearable items category On the basis of event nature and personal information (gender, height, size, skin tone) that it received from ICA. CSA accesses the knowledge base that keeps record of all the items according to gender, color, size, event nature and seasonal stuff. Knowledge base is used predicate logic for the storage and retrieval commodities. Figure 4 shows example which uses rule to store an item into Knowledge base. Similar rules are used for the storage of all accessories.

CSA sends queries to the knowledge base so that it can show list of desiring items. Figure 5 shows the retrieval rule of dresses on the basis of price, color, size and event category e.g. party, wedding, causal etc. Similar rules are used for the suggestion of jewelry, shoes, glasses, hairstyles commodities.

```
STARTRULE "Gents Dress Categories"
IF selected-Category = "DP" /* Dress pent */
AND Waist (DP) <= 32
AND Length <= 42
AND Seansonal_Stuff (DP) = "Cotton"
AND Stuff_Print(DP) = "Lining"
THEN
Store the item as summer wear lining dress pants of medium size with blue color
```

Figure 4 : Rule to Store Dress Pant Items into Gents Dress Categories

3.5 Decision Consultant agent (DCA)

DCA receives a list of selected wearable items from the knowledge base system on the basis of CSA provided information. If there is no match found according to user's specified color then knowledge base itself delivers list of same items in same size but with different colors. DCA gets user image from the user data base and checks all items on user's image and sends that images to the DDA.

3.6 Decision Dispatcher Agent (DDA)

DDA performs two main tasks. (a) Best suitable item decision. DDA receives all images from the DCA and saves them into its own decision support system data base. DSS has capability to make comparisons by using Hamming Distance Algorithm [13] among all images and choose one of them that are more suited to the appearance of the user. Hamming Distance Algorithm uses XOR operator for making comparison between two images. It also sends the images to the converter selector that again converts the images description into text and forwards to the VPA.

VPA transfers text information to the speech synthesizer that informs the user about all images description verbally so that user can share its own choice because may be there is color conflict between user and DSS chosen item. (b) When user is satisfied by the DDA then order placement is also responsibility of DDA. DDA sends the selected items number to the counter system so that they can pack accessories for the user.

In this way, ITAB helps the vision impaired persons to choose best commodities according to their physical appearance and nature of functions. In absence of any human assistant (friend, relative, care attendant) vision impaired person can buy desired accessories with best color combination and matching.

```
STARTRULE "Dress Suggestion"
IF User (Trial_1) = B
AND Requested_Item (B) = "Dress"
AND Gender (B) = "Fair"
AND Height (B) = 42
AND Event_Category (Requested_Item) =  X
AND Requested-Color(X) = "Seagreen"
AND Requested_Stuff  = "Spring"
AND Requested_Size = "Medium"
AND Price_Range = 5000
THEN
Display <-= Selected_Dress_list with seagreen color
Suggest <= Display (other newly fashioned items list with other related shades /colors)
```

Figure 5 : Rule for Dress Suggestion.

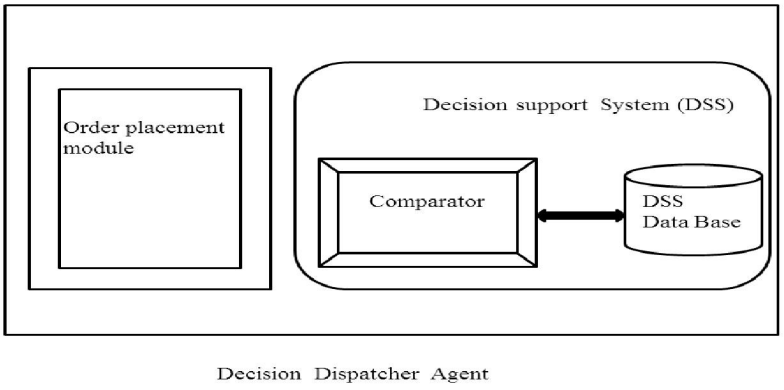

Figure 6 : Decision Dispatcher Agent

4 Case Studies

In this case study we are considering two different scenarios that show the assistance of ITAB for the vision impaired people. Let's consider a shopping mall that is using ITAB software as a shopping assistant for blind people.

Tina a blind girl wants to purchase a party wear. As she enters into the trial room the camera captures image to get her skin tone and facial appearance and sends it to the system. System welcomes Tina and takes personal information like what is your name? Tell me about your gender (male, female), height (either above or below 5.5 feet, or either above or below 6), size (small, medium, large, extra-large), favorite color (red, green, blue, white, sea green, black, skin, purple, magenta, etc.), type of items (dress, shoes, jewelry, hair styles, glasses), seasonal stuff (summer, winter, spring), nature of event (causal , wedding, party , formal) and price range . Tina tells that she is a female with height below 5.5 feet. She needs a sea green party dress in medium size within range of five thousand for spring season.

After taking all necessary information from Tina PVA forwards this information from input buffer to the CSA. CSA activates the desiring speech to text convertor and image to text convertor respectively. ICA extracts all necessary information like gender, height, size, color, item size and price range and send to the CSA so that it can easily interact with the knowledge based that is populated on the basis of item type, seasonal stuff, event nature, size, price range, and color.

ICA sends query to the KB system that shows all the party dresses with medium size and within Tina's price range and sea green color with chiffon, crinkle chiffon stuff. Figure 5 shows the extraction of sea green party dresses from the female portion of accessories from the knowledge base. All the retrieved list of dresses is forwarded to the DCA that gets Tina's image from the user data base and apply all dresses to the Tina's image.

All the images then transfer to the decision support system of DDA that tells about each dress description with new trends like heavy embroidery with quelott, light ribbons work with trouser, stone

work frock with choridar pajama by using the speech synthesizer.

DSS also makes comparisons between all images and tells Tina that the dress with light stone work is looking more beautiful than other dresses. Tina finally selects lightly embroidered dress long shirt with trouser for party.

DDA order place module forwards the selected dress bar code to the counter system and places order for the dress.

Figure 7 : Party Dress Selection for Tina

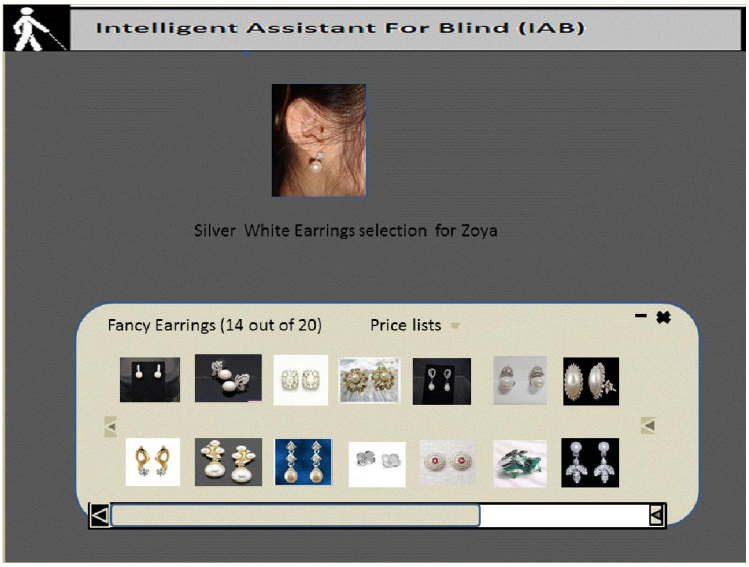

Figure 8 : Earring Selection for Zoya

Zoya is a blind girl that visits shopping mall for the selection of jewelry. After the entrance into the trial room ITAB welcomes Zoya and captures her image for getting her appearance. ITAB takes personal information by asking questions what is your name? Tell me about your gender (male, female), what item do you want to buy(dress, shoes, jewelry ,hair styles, glasses) On the basis of item type ITAB asks more questions like favorite color (red, green, blue, white, sea green, black, skin, purple, magenta, etc.), nature of event (causal , wedding, party , formal) , shape of earrings (round, oval, tree, long, circle, rectangle)and price range . Zoya tells that she is a girl that needs stylish and fancy earrings in small round shape within range of three thousand for formal use in any color. After taking all necessary information from Zoya PVA forwards this information from input buffer to the CSA. CSA activates the desiring speech to text convertor and image to text convertor respectively. ICA extracts all necessary information like gender, item type, size,, item shape and price range and sends to the CSA so

that it can easily interact with the knowledge base that is populated on the basis of item type, seasonal stuff, event nature, size, price range, and color. ICA sends query to the KB system that shows all small size fancy and stylish earrings in round shape within Zoya price range. Figure 7 shows the extraction of fancy earrings from the female portion of accessories in knowledge base. ITAB tells Zoya about the look, material, color combination and price of every earring. Finally system suggests a very stylish silver and off-white pearl earring for Zoya that she can wear with almost all dresses.

5. Conclusion and Future Work

Assistive technology is trying to increase the limited sovereignty of vision impaired persons by introducing new devices and intelligent software assistants. These assistants support blinds in reading, writing, walking, face recognition as well as boost up their confidence level. Still blind people are dependent for the selection of their daily commodities and shopping. Thus, we are also contributing in this era by proposing and implementing the ITAB system that help them in selecting their daily wearable items by using Knowledge based system ,image processing techniques and text to speech technology. A trial room is equipped with hardware support like camera, speaker, microphone, and gait detector. System uses Hamming Trace Transform method and sampling mechanism to extract facial expression and skin tone respectively from user's image. SPOC based speech to text system is used for voice to text conversion. Rules using Predicate logic are used to extract the desiring wearable items from Rule based Knowledge based system. Furthermore, a Decision Support system makes comparisons using Hamming Distance Algorithm between user's best getups. Speech synthesizer provides detail information about each item so that user can easily make decision according to his/her own choice. In this way ITAB facilitates vision impaired person to select their commodities with open choice and independently.

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