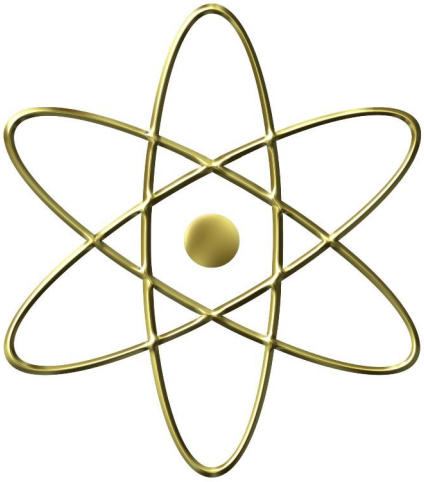
The Journal of American Science



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The Journal of American Science

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3	**Model for Calculating the Concentration of Dissolved Iron Relative to the Final Solution pH and Temperature during Oxalic Acid Leaching of Iron Oxide Ore.** Chukwuka I. Nwoye[1] and Ihuoma E. Mbuka[2] [1]Department of Materials and Metallurgical Engineering, Nnamdi Azikiwe University P.M.B 5025 Awka, Nigeria [2]Department of Materials and Metallurgical Engineering Federal University of Technology, P.M.B 1526 Owerri, Nigeria. chikeyn@yahoo.com **Abstract:** Model for calculating the concentration of dissolved iron (relative to the final solution pH and temperature) during leaching of iron oxide ore in oxalic acid solution has been derived. The model; $\%Fe = 1.1849(\gamma/T)^3$ was found to calculate the concentration of dissolved iron being dependent on the values of the final leaching solution pH and temperature measured during the leaching process. It was observed that the validity of the model is rooted in the expression $(\%Fe/N)^{1/3} = \gamma/T$ where both sides of the expression are approximately equal to 0.2. The maximum deviation of the model-predicted concentration of dissolved iron from the corresponding experimental values was found to be less than 18% which is quite within the acceptable range of deviation limit of experimental results. Concentrations of dissolved iron per unit rise in the solution temperature as obtained from experiment and derived model were evaluated as 0.0011 and 0.0015 $\%/^0C$ respectively, indicating proximate agreement. [Journal of American Science. 2011;7(1):12-18]. (ISSN: 1545-1003). **Keywords:** Model, Dissolved Iron, Solution pH and Temperature, Oxalic Acid, Iron Oxide Ore	Full Text	3
4	**Cytogenetic effect of Insecticide Telliton and Fungicide Dithane M-45 on Meiotic Cells and Seed Storage Proteins of *Vicia faba.*** *Atef A. A. Haiba; Nagwa R. Abd El-Hamid; Elham A. A. Abd El-Hady and Abd El-Rahman M.F. Al-Ansary Department of Genetics and Cytology, Genetic Engineering Division, National Research Center, Dokki, Giza, Egypt. *Atefhaiba@yahoo.com **Abstract:** The genotoxic effects of insecticide Telliton and fungicide Dithane M-45 were examined on meiotic cell divisions and changes in the M2 seed storage protein banding pattern of *Vicia faba* plants. The percentage of abnormal pollen mother cells, (PMCs) increased as the concentration of both pesticides increased. All concentrations and treatment periods of both pesticides, induced a number of chromosomal aberrations in PMCs as stickiness, bridges, laggards, disturbed, micronuclei and multinucleate. A marked change was observed in the M2 *V. faba* seed storage protein banding pattern. These changes included alterations in band intensity, relative mobilities, disappearance of some bands and appearance of new other ones. These results showed that Telliton has more mutagenic effects than Dithane M-45. [Journal of American Science. 2011;7(1):19-25]. (ISSN: 1545-1003). **Key words:** *Vicia faba*, chromosomal abnormalities, insecticide, fungicide and SDS -PAGE protein	Full Text	4

5	**Studies on the uptake of heavy metals by selected plant species growing on coal mine spoils in sub-tropical regions of India** Bandita Deo[1], Gayatri Nahak[2], and R.K.Sahu[2] 1. Regional Plant Resource Center, Nayapalli, Bhubaneswar, Orissa,India 2. Department of Botany, B.J.B (A) College, Bhubaneswar-751014, Orissa, India sahurajani@yahoo.co.in **Abstract:** The accumulation of heavy metals in naturally occurring plants of herbs, shrubs and trees grown on South Bolanda coal mine overburdens in subtropical region of India were illustrated.. The inter-elemental relationships of different parts of five plant species including herbs, shrubs and trees with the coal mine wastes were studied. From the tree species maximum positive correlation was observed for Cu in stem and leaf of *Trema orientalis*. The stem and leaf of *Haldina cordifolia, Diospyrous melanoxylon* and *Ixora arborea* showed positive correlation for Cr, Fe and Cu respectively. Among the shrubs in *Phyllanthus reticulatus*, Cr in stem showed a positive correlation with Cr in leaf. Here among five species of annual herbs, the correlation coefficient for inter elemental variable of whole plant and coal mine spoil for chromium was marked in *Catharanthus roseus*. From the above investigation it was concluded that stabilization of coal mine spoils could be achieved successfully by the plantation of suitable plant species available in native area. [Journal of American Science. 2011;7(1):26-34]. (ISSN: 1545-1003). **Key words:** Coalmine spoils, Heavy metal, Inter-elemental relationship, Overburden Positive correlation	Full Text	5
6	**Credit and money market of the bank of the central Africa States (BEAC)** Ndjedanem Demtade Nadingar[1], Chen Shuwang yang [1] China University of Geosciences (Wuhan) 388 Lumo Road, Wuhan, P.R. China Postcode: 430074. alafi2004@yahoo.fr **Abstract:** In a context of world economic crisis, our article on the credit and money market aim to emphasize the influence of the bank of the States of Africa on the saving in each one of its members in general and on Chad in particular through the service of credit and money market. [Journal of American Science. 2011;7(1):35-39]. (ISSN: 1545-1003). **Key words:** BEAC, Credit, Money Market, Interbank market, obligatory reserves	Full Text	6

7	**Women's Empowerment for Rural Development** Fatemeh Allahdadi Dept. of Organizational and Industrial Psychology, Islamic Azad University, Marvdasht Branch faaref@yahoo.com Abstract: The main objective of this study provides a strategy for women's empowerment for rural development. Empowerment can enable women to participate, as equal citizens, in the economic, political and social sustainable development of the rural communities. The findings outlined in this paper suggest that, designed and implemented in ways that meet rural women's diverse needs, community participation processes that can be important to facilitating social, technological, political and psychological empowerment in terms of rural development. The findings of this investigation can assist rural developers in the implementation of community development strategies based on women's empowerment. **[**Fatemeh Allahdadi. **Women's Empowerment for Rural Development.** Journal of American Science 2010;7(1):40-42]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** women's empowerment, rural development, local development	Full Text	7
8	**Inhibitory effects of two indigenous plant extracts (*Zingiber officinale* and *Ocimum gratissimum*) on post harvest yam (*Dioscorea rotundata Poir*) rot, *in vitro*.** **Ijato James Yeni** Department of Plant Science, Faculty of Science, University of Ado Ekiti, P.M.B 5363, Ekiti State, Nigeria. E-mail: jamesyeni@yahoo.com; GSM: 08067335124 **Abstract:** Cold water and ethanol extracts of two fungicidal plants (*Zingiber officinale* and *Ocimum gratissimum*) were screened for their *in vitro* effects on rot fungi of yam using 60 and 80% aqueous extract and 20 and 30% ethanol extract of each concentration. The two concentrations of aqueous and ethanol extracts were found to have inhibitory effects on all the rot fungi isolated from yam, 80% *a*queous extract of *Zingiber officinale* inhibited *Fusarium oxysporum* to 66.70%, 80% aqueous extract of *Ocimum. gratissimum* inhibited *Botrydioploidia theobromae* to 60.00% also73.33% inhibition of *Aspergillus flavus* was recorded using 30% ethanol extract of *Zingiber officinale*, the same concentration of *Ocimum gratissimum* inhibited *Aspergillus niger* to 70.00%. Both aqueous and ethanol extract of *Zingiber officinale* and *Ocimum gratissimum* had potential inhibitory effect on all the rot fungi. [Ijato James Yeni. **Inhibitory effects of two indigenous plant extracts (*Zingiber officinale* and *Ocimum gratissimum*) on post harvest yam (*Dioscorea rotundata Poir*) rot, *in vitro*.** Journal of American Science 2011;7(1):43 - 47]. (ISSN: 1545 - 1003). http://www.americanscience.org. **Key word** *In vitro, Zingiber officinale, Ocimum gratissimum*, rot fungi, yam	Full Text	8

9	Testicular maturation and reproductive cycle in mudskipper, *Periophthalmus papilio* (Bloch and Schneider 1801) from Lagos lagoon, Nigeria **LAWSON, Emmanuel O.** Department of Fisheries, Faculty of Science, Lagos State University, Ojo. P.O. Box 001, LASU Post Office Box, Lagos, Nigeria ollulawson@yahoo.com **Abstract:** A study was carried out on mudskipper, *Periophthalmus papilio* from Lagos lagoon, Nigeria to determine its testicular maturation and reproductive cycle. *P. papilio* is a commercial valued fish in Nigeria as food for man and baits in capture fisheries, making its population in Lagos lagoon to be threatened. Therefore, conservation of its fishery from overfishing and exploitation is urgently required. A total of 796 male individuals were captured with non return valve traps between July 2004 and July 2006 from mangrove swamps of Lagos lagoon. They measured between 37 and 180 (104.83±25.57) mm TL and weighed 1.5 – 60.9 (18.60±10.65) g BW respectively. The testes were morphologically examined by naked eye and processed by standard histological techniques. ICES, BITS and IBTS scales and Bucholtz manuals were employed in the classifications of its maturity and gonadal stages. Seven reproductive stages were encountered in the study viz. immature, immature and developing, ripening, ripe, ripe running, spent and recovering-spent. The reproductive cycle included pre-spawning, spawning and post-spawning phases. The testicular maturation and reproductive cycle in mudskipper, *P. papilio* though with modifications were similar to what obtained in other teleosts. The GSI values ranged between 0.01 and 0.48 (0.132±0.165) i.e. less than 0.48% of the body weight was converted to development of testes. GSI values were at different peaks in July (0.23±0.016) and September (0.30±0.13%) 2004; May (0.198±0.004) and October (0.097±0.009%) 2005; and January (0.865±0.12), April (0.122±0.009) and July (0.145±0.016%) 2006 indicating the species as a multiple and synchronous spawner in Lagos lagoon. The study therefore provides the basic life history information on *P. papilio* through an objective approach in the assignment of maturity stage, using histological technique and macroscopic evaluations of the testes. [LAWSON, Emmanuel O. Testicular maturation and reproductive cycle in mudskipper, *Periophthalmus papilio* (Bloch and Schneider 1801) from Lagos lagoon, Nigeria. Journal of American Science 2011;7(1):48-59]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words:** Gonadosomatic index, spawning, spermatocyte, spermatid, spermatozoon, mudflat	Full Text	9

10	**Application of an Artificial Neural Network Model to Rivers Water Quality Indexes Prediction – A Case Study** Hossein Banejad [1], Ehsan Olyaie [1] [1.] Department of Water Engineering, Faculty of Agriculture, Bu-Ali Sina University of Hamedan, Iran Hossein_banejad@yahoo.com **Abstract:** Taxonomic Recent trends in the management of water supply have increased the need for modeling techniques that can provide reliable, efficient, and accurate representation of the nonlinear dynamics of water quality within water distribution systems. Since artificial neural networks have been widely applied to the nonlinear transfer function approximation, in this study we present an empirical multi layer perceptron neural network to estimate water quality indexes (BOD, Do) in Morad Big River in the western part of Iran. In this paper, the information and data including 10 monthly parameters of water quality in the Hamedan Morad Big River in duration of one year and six stations were used for modeling biological oxygen demanded (BOD) and dissolved oxygen (DO) as indices affecting water quality. To validate the performance of the trained ANN, it was applied to an unseen data set from a station in the region. Performance of the model was evaluated by statistical criteria includes correlation coefficient (r), root mean square error (RMSE) and mean absolute error (MAE). In the optimum structure of neural network correlation coefficient for BOD and DO are 0.986 and 0.969, also root mean square error are 8.42 and 0.84 respectively. The results show the identified ANN's great potential to simulate water quality variables. [Hossein Banejad, Ehsan Olyaie. Application of an Artificial Neural Network Model to Rivers Water Quality Indexes Prediction – A Case Study. Journal of American Science 2011;7(1):60-65]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Artificial Neural Networks; Predicting; Water Quality Index; BOD; DO	Full Text	10
11	**Favorable Content of Sustainable Agriculture Extension Programs In Khouzestan Province of Iran** Ahmad Reza Ommani Department of Agriculture, Islamic Azad University Shoushtar Branch ommani75451@yahoo.com **Abstract:** The purpose of research was identify favorable content of sustainable agriculture extension programs in Khouzestan province of Iran. A sample of 79 respondents was selected through simple random sampling technique. A survey study was applied as a methodology of research work. Data were collected using a structured questionnaire that addressed to evaluate agricultural extension experts' responses regarding the necessity of attention on each extension system content to accomplish sustainable agriculture in Khouzestan province of Iran. For determining the validity of questionnaire, the face and content validity was used. Cronbach's alpha was used to measure reliability of the instrument, which was 0.80 and showed the instrument reliability. Descriptive findings revealed that "Food security", "Integrated management", "Biological control practices", "Quality of crops" and "Conservation practices" were the first contents for extension system toward sustainability, respectively. According to factor analysis, the contents of extension system for supporting of sustainable agriculture were categorized into three main components, which have been named *Natural conservation, Human health and Economic contents*. The obtained results from the factor analysis revealed that the three mentioned factors explained 75.231% of the variation of extension content for supporting of sustainable agriculture in agriculture. [Ahmad Reza Ommani. Favorable Content of Sustainable Agriculture Extension Programs In Khouzestan Province of Iran. Journal of American Science 2011;7(1):66-70]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Content of extension; Agricultural Sustainability	Full Text	11

12	[Journal of American Science 2011;7(1):71-79]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 9	Full Text	12
13	**GC/MS Determination of Bioactive Components of *Murraya koenigii*** [1]Hema R., [2]S. Kumaravel and [3]K. Alagusundaram [1]Senior Research Fellow, Department of Food Quality and Testing, IICPT [2]Scientist, Department of Food Quality and Testing, IICPT [3]Director, Indian Institute of Crop Processing Technology (IICPT), Thanjavur, TamilNadu, India e-mail: hema.scientist@gmail.com **Abstract:** In this study, the bioactive components of *Murraya koenigii* leaves have been evaluated using GC/MS. The chemical compositions of the ethanol extract of *Murraya koenigii* were investigated using Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. GC/MS analysis of ethanol extract of *Murraya koenigii* revealed the existence of 1-Methyl-pyrrolidine-2-carboxylic acid (69.00%), Ethyl à-d-glucopyranoside (13.36%), Isolongifolene, 4,5-dehydro- (3.68%), ç-HIMACHALENE (2.88%), 1,2-Ethanediol, monoacetate (2.79%) 1,2-Benzenedicarboxylic acid, diisooctyl ester (2.55%). The results of this study offer a platform of using *Murraya koenigii* as herbal alternative for the current synthetic antimicrobial agents. [Hema R., S. Kumaravel and K. Alagusundaram. **GC/MS Determination of Bioactive Components of *Murraya koenigii***. Journal of American Science 2011;7(1):80-83]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words**: *Murraya koenigii*, GC/MS, Bioactive components	Full Text	13

14	**Stainless steel implantation-induced changes in surface characteristics, corrosion resistance and hemato-biochemical parameters of male rat** Sahar A.Fadl-allah[1, 3 *], Q. Mohsen[1] and Nahla S. El-Shenawy[2, 4] [1]Materials and Corrosion Lab (MCL), Faculty of Science, Taif University, Taif, K.S.A [2]Zoology Department, Faculty of Science, Taif University, Taif, K.S.A [3]Chemistry Department, Faculty of Science, Cairo University, Giza, Egypt [4]Zoology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt saharfadalla@hotmail.com **Abstract:** In this study the physiological solution effect on corrosion resistance and surface characteristics of stainless steel has been studied *in vitro* by electrochemical measurements and microstructure characterization of the surface. All studies were carried out using phosphate buffer saline (PBS) as a simulated physiological solution. Potentiodynamic polarization results indicated a considerable shift of pitting potential of the specimen in the noble direction after14 days of immersion in PBS. As evidenced by electrochemical impedance spectroscopy (EIS), the effect of long immersion of stainless steel in physiological solution on the passive film stability was proved. The surface structure and composition before and after immersion in PBS were then characterized by means of scanning electron microscopy (SEM) with electron diffraction X-ray analysis (EDX) techniques. The electrochemical measurements and fitting parameters showed that the passive film formed on stainless steel decreased the corrosion currents densities (I_{corr}) and the constant phase elements (*CPE*), as simultaneously increased the values of polarization or charge transfer resistance (R_{ct}) of stainless steel in simulated physiological solution. The physiological and histological effects of pitting corrosion of stainless steel metal were studied after 14 days of post-implantation in the tibiae of Sprague-Dawley male rats. The stainless steel implantation caused a slightly increased in blood haemoglobin, total erythrocytes count and packed cell volume, and significantly decreased total leukocyte count. All the hepatic enzymes activities of a separate aminotransferase, alanine aminotransferase, alkaline phosphatase and lactate dehydrogenase were significantly decreased. The activity of glutathione S-transferase and the level of lipid peroxidation were significantly increased while hepatic glutathione was significantly decreased. The toxicity of stainless steel in implanted rat could be related to the biodegradation of the alloy and releasing of Fe, Mn, Ni and Cr in the rat tissue as indicated by the *in vitro* study. The bone regeneration was observed at the surface near the stainless steels implants after two weeks of implantation. [Sahar A.Fadl-allah, Q. Mohsen and Nahla S. El-Shenawy. **Stainless steel implantation-induced changes in surface characteristics, corrosion resistance and hemato-biochemical parameters of male rat**. Journal of American Science 2011;7(1):84-91]. (ISSN: 1545-1003). http://www.americanscience.org. ***Keywords*:** Impedance spectra; Pitting corrosion; Scanning electron microscope (SEM); Electron diffraction X-ray (EDX) analysis; Lipid peroxidation; Glutathione; Toxicity; Bone repair	Full Text	14

15	**A Review of the Problems Faced by AIOU Regional Centers in Pakistan** Tariq Mehmood [1] Zahoor ur Rehman Tariq Jamil Preston University Al-Khawarizmi Institute of Computer Science Sultan Qaboos University Islamabad, Pakistan University of Engineering & Technology Oman. tariq_619219@yahoo.com Lahore, Pakistan. tjamil@squ.edu.om xahoor@uet.edu.pk **Abstract:** The objective of the study was to investigate the problems faced by the regional centers of Allama Iqbal Open University (AIOU) Pakistan. For the purpose of collection of data, a questionnaire was developed and the data collected through the questionnaire were tabulated, analyzed, and interpreted. Major findings of the study reveal that the major problems faced by AIOU regional centers staff are the limited frequency of capacity building workshops, shortage of transport facility, and the absence of purpose-built infrastructures for the regional centers. Overcoming these deficiencies at the regional centers will result in better working environment at these centers and hence yield to overall better performance. [Tariq Mehmood, Zahoor ur Rehman, Tariq Jamil. **A Review of the Problems Faced by AIOU Regional Centers in Pakistan**. Journal of American Science 2011;7(1):92-99]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Allama Iqbal Open University, distance education, regional centers, problems	Full Text	15
16	**Comparison between Outer Membrane Protein Profile of Fluoroquinolones Sensitive and Resistant *P. aeruginosa* Isolated from Egyptian Patients** Eman Shams-Eldin [*1], Salah Abdalla [2], Alaa El-Dein Mahmoud Shawki [3] and Abeer Galal-Eldin [4] Ministry of Health, Egypt [1], Department of Microbiology and Immunology, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt[2], Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo University, Cairo, Egypt [3], Department of Microbiology and Immunology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt [4] [*]eshamseldin@yahoo.com **Abstract:** *Pseudomonas aeruginosa* is an important opportunistic pathogen that infects immunocompromised hosts and is characterized by its natural resistance to a variety of antimicrobial agents. The purpose of this study was the assessment of the fluoroquinolones resistance level among *P. aeruginosa* clinical isolates, furthermore to compare between the outer membrane protein profile of fluoroquinolones susceptible and resistant isolates of *P. aeruginosa* using Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) technique. Sixty five (43%) were identified as *P. aeruginosa* by conventional culture techniques. MIC of ciprofloxacin, norfloxacin and levofloxacin against pseudomonal isolates were determined by twofold agar dilution technique. Only about 39%, 40% and 42% of these isolates were resistant to ciprofloxacin, levofloxacin and norfloxacin, respectively. Profile of outer membrane protein fraction of the fluoroquinolones resistant isolates showed an additional band with an approximate molecular weight of 50-54 kDa. In conclusion, overproduction of outer membrane protein of approximate molecular weight 50-54 kDa in *P. aeruginosa* was associated with fluoroquinolones resistance. [Eman Shams-Eldin, Salah Abdalla, Alaa El-Dein Mahmoud Shawki and Abeer Galal-Eldin. **Comparison between Outer Membrane Protein Profile of Fluoroquinolones Sensitive and Resistant *P. aeruginosa* Isolated from Egyptian Patients**. Journal of American Science 2011;7(1):100-104]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** *Pseudomonas aeruginosa*- fluoroquinolones- outer membrane protein- SDS_PAGE	Full Text	16

17	**_In-vivo_ and _in-vitro_ Prediction of the Efficiency of Nano-Synthesized Material in Removal of Lead Nitrate Toxicity** **Eman I. Abdel-Gawad[*1] and Sameh A. Awwad[2]** [1]Radioisotopes Department, Atomic Energy Authority, [2]Egyptian Army Forces, Egypt dr.eman_57@hotmail.com[*] **Abstract:** Due to large grain sizes, the biological properties of the conventional hydroxyapatite (HAp) is limited to a great extent. Progresses in nanotechnological approaches now allow the fabrication of nano-HAp. In this study, firstly, the characters of nano-hydroxyapatite gel was described and the interaction performance of the formed gel with lead nitrate $Pb(NO_3)_2$ *in vitro* was identified. Then, the biological efficiency of nano-HAp gel against $Pb(NO_3)_2$ toxicity *in vivo* was introduced. A polymeric matrix route was selected to synthesis nano- composite hydroxyapatite gel. The formed gel characterized using FTIR, XRD, SEM, TEM. Various volumes of the produced nano-HAp gel (10, 20, 30, 40, 50 and 60 µl) was adding to 4 ml of ECS solution. The clear supernatant was separated and analyzed by ICP-MS. The results showed a successful removal of lead ions by formed gel. A single dose of intravenous nano-hydroxyapatite at a level of 150 and 300 mg/kg b.w. was injected to male rats following intraperitoneal 93mg/kg b.w. (LD_{50}) of lead nitrate $Pb(NO_3)_2$. The results revealed that nano- HAp composite had the ability to alleviate lead nitrate toxicity, to a great extent, in serum antioxidant status, liver and kidney function as well as corticosterone and calcium levels but phosphorus value was not affected among the all treated groups. However, most successful results were attributed to the treatment with high dose of formed nano-HAp particularly after 48 h more than the treatment with low dose. Histopathological observations confirmed the biochemical results, since nano-HAp into rats evident the recovery of lead nitrate cytotoxicity in liver and kidney cells. [**Eman I. Abdel-Gawad[*1] and Sameh A. Awwad.** *In-vivo* and *in-vitro* Prediction of the Efficiency of Nano-Synthesized Material in Removal of Lead Nitrate Toxicity. Journal of American Science 2011;7(1):105-119]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Nano-HAp, lead nitrate, antioxidant status, liver enzymes, kidney functions , corticosterone	Full Text	17
18	**Silver nitrate staining improves visual analysis of daily otolith increments** Trika L. Gerard[1] (corresponding Author), and Estrella Malca[2] [1]NOAA Southeast Fisheries Science Center, 75 Virginia Beach Drive, Miami, FL 33149, USA, 305-361-4493, 305-365-4103 (Fax). Trika.Gerard@noaa.gov [2]Cooperative Institute for Marine and Atmospheric Studies, University of Miami, 4600 Rickenbacker Cswy, Miami, FL 33149, USA, 305-361-4295, 305-361-4103 (Fax). Emalca@rsmas.miami.edu **Abstract:** Sagittal otoliths in juvenile to sub-adult (62mm-150mm standard length) gray snapper (*Lutjanus griseus*) were analyzed using a modified staining method. Daily growth increments from transversely sectioned otoliths were stained using silver nitrate and fixed using sodium thiosulfate. Stained otoliths showed a noticeable improvement in the resolution of daily increments compared to those not stained. This procedure lends to the enhanced visualization of daily rings and has the potential to be a timely, yet efficient, technique for age and growth analysis of calcium carbonate structures. [Trika L. Gerard, Estrella Malca. Silver nitrate staining improves visual analysis of daily otolith increments. Journal of American Science 2011;7(1):120-124]. http://www.americanscience.org. **Keywords:** silver nitrate, staining, otolith, daily increment, von Kossa	Full Text	18

19	**Arsenic Toxicity in the Irrigation Water-Soil-Plant System: A Significant Environmental Problem** Hossein Banejad [1], Ehsan Olyaie [1] [1] Department of Water Engineering, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran Hossein_banejad@yahoo.com **Abstract:** Environmental pollution is a major global concern. When sources of water pollution are enumerated, agriculture is, with increasing frequency, listed as a major contributor. One of the major factors determining uptake and toxicity to plants is the form of arsenic (As). Naturally occurring arsenic in groundwater of sedimentary aquifer has emerged as a global problem, and issue of major environmental concern. It is released and contaminated in agricultural soil by natural weathering, industrial production and mining. However, the same water resources are used extensively for irrigation purposes throughout the region. The two most important forms, As (V) and As (III), are taken up by completely different mechanisms. Uptake, accumulation and toxicity vary within and between plant species. In general, more As in the soil leads to higher concentrations in plants, but this depends on many factors. It is recommended to initiate an integrated program to quantify the scale of the problem in combination with the development of a water-soil-plant quality monitoring system for land degradation in agro-ecosystems. This should not only include As, but a range of physical, chemical (nutrients and contaminants) and biological parameters. Further, management options to prevent and mitigate As contamination need to be explored. [Hossein Banejad, Ehsan Olyaie. **Arsenic Toxicity in the Irrigation Water-Plant Environment: A Significant Environmental Problem.** Journal of American Science 2011;7(1):125-131]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Arsenic, Toxicity, Irrigation, Water-Soil-Plant System, Environment	Full Text	19
20	**Improvement of Oxidation Stability of Mineral Oil using Jojoba Oil** **Elham A. Eissa*, Renee I. Abdallah and Afaf R. Taman** Egyptian Petroleum Research Institute, Cairo, Egypt **el_awadi@yahoo.com** **Abstract:** The production of insulating mineral oil from naphthenic fraction (b.r. 300-420^{o}C) was carried out by furfural solvent extraction. The refined oil and its binary mixtures with jojoba oil at different concentrations 20, 50, and 80 vol % have been employed as synthetic insulating oil in a wide variety of electrical equipment. The physico-chemical properties of the refined oil as well as the electrical properties of the mixtures were determined. The oxidation stability of original oil, refined mineral oil and its binary mixtures with jojoba oil with different concentrations was studied. The stability of oxidation by adding different concentrations of 2,6,-di-tertiarybutyl phenol inhibitor to binary mixture containing 20 vol % jojoba oil was studied. It is found that the maximum stability is obtained by adding 2 wt % of inhibitor. [Elham A. Eissa, Renee I. Abdallah and Afaf R. Taman. **Improvement of Oxidation Stability of Mineral Oil using Jojoba Oil.** Journal of American Science 2011;7(1):132-137]. (ISSN: 1545-1003). http://www.jofamericanscience.org. **Key Words:** Mineral oils, Oxidation stability, Jojoba oil, Inhibitor, Electrical properties	Full Text	20

21	**Mapping water quality of Burullus Lagoon using remote sensing and geographic information system** Mohamed E. Hereher; Mahmoud I. Salem and Dina H. Darwish Department of Environmental Sciences, Faculty of Science at Damietta, Mansoura University, Egypt. **dina_200777@yahoo.com** **Abstract:** The present study aims to utilize remote sensing and a geographic information system (GIS) for mapping surface conditions of the Burullus Lagoon, Egypt as a proxy to water pollution. Spatial distribution of suspended matter, nitrogen, phosphorous, chlorophyll, dissolved oxygen, water temperature, salinity, depth, lead, copper, cadmium, clay, and sediment organic carbon has been applied. A Landsat image from the Enhanced Thematic Mapper plus (ETM+) sensor acquired in June 2006 was processed based on a band by band as well as band rationing. Cartographic maps were generated depending on the correlation between the measured parameters and the radiance values of the ETM+ image. Parameters not correlated with the satellite image data have been processed through spatial analysis and interpolation technique using GIS. Results showed that the eastern and southern sections of the lagoon, which receive drainage wastewater, are more polluted than the northern and western sections of the lagoon. The study confirms that remote sensing coupled with GIS could afford an integrated scheme for mapping water quality. [Mohamed E. Hereher; Mahmoud I. Salem and Dina H. Darwish. Mapping water quality of Burullus Lagoon using remote sensing and geographic information system. Journal of American Science 2011;7(1):138-143]. (ISSN: 1545-1003). **Keywords:** Mapping; water quality; Burullus Lagoon; geographic information system	Full Text	21
22	**Path Analysis of Direct and Indirect Effect of Statistical literacy on Applying Proper Statistical Test (Case Study of agricultural extension and education graduated students)** Sahar Dehyouri [1], Iraj Malek Mohammadi [2], Seyed Mahmood Hosseini [2], Seyed Mehdi Mirdamadi[1] 1. Department of Agricultural Extension and Education, Science and Research branch, Islamic Azad University, Tehran, Iran, dehyouri.s@gmail.com 2. Department of Agricultural Extension and Education, Karaj campus, Tehran University, Karaj, Iran **Abstract:** Research methods, statistical analysis and domination on subject are essential for a rich dissertation and thesis to be developed. The main goal of this study was to obtain the perception of the agricultural extension and education graduated students about their statistical literacy, reasoning and thinking according to standard tests and to trace thematic evolution (content analysis) of dissertations and thesis done by the same graduated students according to sequential statistics analysis approach (SSAA). To this end, the study analyzed 315 thesis and dissertation to understand, how and to what extent, proper and mix statistical methods are applied to achieve realistic outcomes. In the other hand, 115 questionnaires were fulfilled, containing statistical standard tests about statistical literacy, reasoning, thinking, attitude, content knowledge and principal component of statistics learning. According to the path analysis results, the statistical attitude (total effect=0.80) had the most effect (direct and indirect effect) on applying statistical methods. **[**Sahar Dehyouri, Iraj Malek Mohammadi, Seyed Mahmood Hosseini, Seyed Mehdi Mirdamadi. **Path Analysis of Direct and Indirect Effect of Statistical literacy on Applying Proper Statistical Test (Case Study of agricultural extension and education graduated students).** Journal of American Science 2011;7(1):144-153]. (ISSN: 1545-1003). http://www.jofamericanscience.org. **Keywords:** statistical literacy, statistical reasoning, statistical thinking, sequential statistical analysis approach	Full Text	22

23	**Study of the nutritional value of Persian Gulf squid (*Sepia Arabica*)** Forough papan, Ashraf Jazayeri, Hussein Motamedi, Soghra mahmoudi asl Shahid Chamran University of Ahwaz, IRAN Corresponding Arthur: jazayeriashraf@yahoo.com **Abstract:** Cephalopodan are a group of mollusks that have substantial geographical distribution .Squid have largest fisheries value between Cephalopoda In the world. In the Persian Gulf and Oman Sea are also squid. Due to good taste and friendly meat market, exports this species has three million dollars Currency returns in year1386. Fish meat there are the unique characteristics, including high protein content, unsaturated fatty acids (EPA, DHA), vitamins and minerals thus Fish consumption in the diet is essential. Marine biologists have extracted the new combination of some aquatic that has significant effects in prevent and treat certain illnesses. Information about the Persian Gulf is very limited in this study the nutritional value of squid was investigated. Results showed that this species, with17 percent protein and 8.9 percent fat, having high nutritional value. To protect these stocks should pay more attention to it. **[**Forough papan, Ashraf Jazayeri, Hussein Motamedi, Soghra mahmoudi asl**. Study of the nutritional value of Persian Gulf squid (*Sepia Arabica*).** Journal of American Science 2011;7(1):154-157]. (ISSN: 1545-1003). http://www.jofamericanscience.org**.** **Keywords:** squid, Persian Gulf, nutritional value, sepia Arabica	Full Text	23

24	**Effect of Some Chemical Compounds on Sedimentation Rate of Different Yeast Strains** Laila M. Abdelaty, Wedad E. Eweda, E. M. Ramadan and A. J. Al-Waraquiy. Department of Agric. Microbiology, Fac. Agri Ain Shams University , Shubra El-Khima,Cairo , Egypt. **rfr2000@live.com** **Abstract:** Heavy metal pollution represents an increasing problem in industrialized as well as developing countries. Yeast cells are capable to accumulate these pollutant from different environments. In this investigation, eight baker's yeast strains were collected from different Egyptian markets. The source of these yeast strains were Misr Yeast, Alinson, Vahine professional, Fermipan, Hollandia Saf–instant, H.u.G and Pakamaya. These strains were grown on basal medium or in molasses medium to determine their efficiency in the bioaccumulation of some metals. The sedimentation measurement was carried out at different salt solutions and different times intervals. The results clearly indicated that SnCl2 followed in descending order by Pb (CH3CooH)2 and AgNO3 were the most effective compounds in increasing the rate of sedimentation of all the tested yeast strains. In contrast; the lowest Figures were recorded with KH2PO4 ,FeCl3 , NiSO4,Co(NO3)2,CaSO4,MgSO4 , Zn SO4, Al2(So)4 and Co CL 2. Other minerals showed a moderate sedimentation capability. It can be stated that yeast cells have a considerable capability to uptake Zinc and iron from the growing medium whereas, manganese showed moderate capability. The lowest values were observed in2the case of copper and lead. *Saccharomyces cerevisiae* can be used as a bioremediation agent for removing heavy metals from the surrounding environment due to its high uptake capacity, taking in consideration that it must be economically competitive with existing technologies. **[**Laila M. Abdelaty, Wedad E. Eweda, E. M. Ramadan and A. J. Al-Waraquiy. **Effect of Some Chemical Compounds on Sedimentation Rate of Different Yeast Strains.** Journal of American Science 2011;7(1):158-162]. (ISSN: 1545-1003). http://www.jofamericanscience.org. **Keywords:** Effect; Chemical Compound; Sedimentation Rate; Yeast Strain	Full Text	24
25	**Evaluate Area for Very Large Integrated Digital Systems Based on Bandwidth Variation** Afshin Shaabany [1], Fatemeh Jamshidi [1] [1] Islamic Azad University, Fars Science and Research Branch, Shiraz, Iran afshinshy@yahoo.com, Fjamshidi59@yahoo. com **Abstract:** In this paper, Network on Chip is used as an alternate approach for very large integrated digital systems (System on chip) that is based on bus communications and IP interconnections. This approach has solved some problems like scalability that buses encounter them. One of the basic steps in this approach is correct simulation of NoC implementation; moreover, simulation design operability and perform ability require its synthesizability. Designing and implementation of NoC communication are presented in this work. Finally, bandwidth variation effect on area requirements is evaluated, and area requirements changing due to these alternations will be discussed and explained. [Afshin Shaabany, Fatemeh Jamshidi. Evaluate Area for Very Large Integrated Digital Systems Based on Bandwidth Variation. Journal of American Science 2011;7(1):163-169]. (ISSN: 1545-1003). http://www.jofamericanscience.org. **Keywords:** Network on Chip, IP interconnection, bandwidth variation effect, scalability, perform ability	Full Text	25

26	**Changing of Self-Care Behavior by Practicing 12-Step Program among Codependents in Iran** Zahra Ajri [1], Shatar Sabran* [1] [1.] Department of Community Development, Faculty of Human Ecology, University Putra Malaysia, Malaysia z.ajri@yahoo.com; * shatar@putra.upm.edu.my **Abstract:** Promoting positive sense of self and taking care of self among people are important factors in order to achieve health promotion in every community. As self-forgetting is special character among codependents, so this study aims to find differences of self-care behavior by comparing families of addicts/alcoholics who practice the "12-step program" and who do not. In other words, this study investigates whether "12-step program" can empower families of addicts/alcoholic to change their self-care style or not. Theory of empowerment is the key theory to conduct this study. The findings of this study indicate that "12-step program" is effectiveness program to enable codependents to having positive self-image. In other words, independent samples t-test reveals that codependents who practice the "12-step program" take care of themselves more than another group who did not practice this program. [Zahra Ajri, Shatar Sabran. **Changing of Self-Care Behavior by Practicing 12-Step Program among Codependents in Iran**. Journal of American Science 2011;7(1):170-173]. (ISSN: 1545-1003). http://www.jofamericanscience.org. **Keywords:** 12-Step program; Addiction; Al-Anon & Nar-Anon; Codependency; Families of Addict; Self-care	Full Text	26
27	**Role of *Atherina* Species in Transmitting some Bacterial Diseases to Human** Mohamed E. M. Mohamed, Maysa A.I. Awadallah*, Magda A. Amin, and Rasha M. M. Abou-Elez Department of Zoonoses, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt maysavet@hotmmail.com* **Abstract:** A total of 530 samples (300 from fresh water marine *Atherina*), 130 samples from water used for preparation of Atherina fish for selling, and 100 hand swabs from their handlers) were collected from randomly selected markets from 3-localities in Sharkia governorate, Egypt. All samples were examined for the presence of *Staphylococcus* species and *Enterobacteriaceae*. Moreover, the effectiveness of freezing, salting, and commercial vinegar (5% acetic acid) treatment on the survivability of *Staphylococcus* spp. and *Enterobacteriaceae* in *Atherina* fish was also evaluated. Results revealed *S. aureus* were detected in 65.7% of the surface swabs and 35.7% of muscle samples of fresh water *Atherina* fish. The prevalence of *S. aureus* in the surface swabs and muscle samples of marine *Atherina* fish were 62.2% and 25.6%, respectively. *Enterobacteriaceae* isolated from surface swabs of *Atherina* fish were; *E. coli* (5.33%), *Kl. Oxytoca* (7%), *Kl. pneumoniae* (5.7%) *Ent. cloacae* (5%), *P. vulgaris* (9%), *P. mirabilis* (6.3%), *Sh. sonnei* (1.7%), *Cit. freundii* (5%), *Cit. koseri* (6%), *Pantoea agglomerans* (38.3%), *Hafnia alvei* (1.7%), *M. morganii* (2.3%), and unidentified spp. (8.7%). The percentages of isolation of the previous species from muscle samples of *Atherina* fish were 0.7, 2.3, 1.3, 1.7, 5, 3.7, 0.7, 1.7, 2.7, 24.7, 1.3, 1.3, and 3.7, respectively. The prevalence of *S. aureus* was 53.1% in water samples used for preparation of fish for selling. *Enterobacteriaceae* isolated from water samples were *E. coli* (6.15%), *P. mirabilis* (7.7%), *P. vulgaris* (11.5%), *Ent. Cloacae* (7.7%), *Cit. freundii* (6.15%), *Cit. koseri* (6.9%), *Kl. pneumoniae* (7.7%), *Kl. oxytoca* (9.2%), *Pantoea agglomerans* (30.7%), *Hafnia alvei* (2.3%), *M. morganii* (3.1%), *Sh. sonnei* (1.5%), and unidentified species (3.8%). *S. aureus* was isolated from 73 hand swabs. *Enterobacteriaceae* isolated from hand swabs were *E. coli* (5%), *P. vulgaris* (8%), *P. mirabilis* (5%), *Kl. Pneumonia* (6%), *Kl. Oxytoca* (7%), *Ent. Cloacae* (6%), *Cit. freundii* (4%), *Cit. koseri* (5%), *Pantoea agglomerans* (36%) and unidentified species (18%). Ten representative biochemically identified *E. coli* isolates (4 from *Atherina* fish, 3 from water used for preparation of fish for selling, and 3 from hand swabs of fish handlers) were identified as O_{128} (2-strains), O_{114} (strain), and O_{136} (strain) from *Atherina* fish, O_{26} (strain), O_{111} (strain) and untyped strain from hand swabs of fish handlers. However, all isolates from water samples were O_{128}. The survivability experiment revealed that all muscle samples were negative for all bacteria species growth	Full Text	27

	from the 1st week of freezing. After 1st week from freezing, all *Enterobacteriaceae* were continued to isolate (1:4 each) from the surface swabs of the 4 examined samples. On the other hand, *S. aureus* was continued to isolate at a rate of 4:4 . All *Enterobacteriaceae* except *P. mirabilis* (each with 1:4), *S. aureus* (4:4), Coagulase negative *Staphylococcus* spp. (1:4) were continued to isolate after the 2nd week from freezing. The isolated species after the 3rd week of freezing were *Kl. oxytoca, Pantoea agglomerans*, and un-identified species (1:4 each), and *S. aureus* (4:4). *Pantoea agglomerans*, *un-identified* species and *S. aureus* were continued to isolate after 4th week. The un-identified species (1:4) and *S. aureus* (4:4) were continued to isolate until the week 13 from freezing. *Kl. oxytoca, P. vulgaris, P. mirabilis* (1:4, each) were isolated from surface swabs of fresh water *Atherina* fish salted in NaCl solution (25%). Moreover, *Pantoea agglomerans* and *S. aureus* were isolated with ratios of (2:4) and (4:4) of the same samples, respectively. On the other hand, the bacterial spp isolated from the muscle samples of fish salted at NaCl 25% were *Kl. oxytoca, Pantoea agglomerans* (1:4, each). All samples salted in 50% and 75% NaCl solution were negative for the presence of *Enterobacteriaceae* from the 1st week and for the whole period of the experiment. *E. coli* was continued to isolate until the 6th hours of treatment but stop to grow after 7 hours from vinegar treatment. *S. aureus* was negative in all treated samples from the 1st hour of treatment. [Mohamed E. M. Mohamed, Maysa A.I. Awadallah, Magda A. Amin, and Rasha M. M. Abou-Elez. **Role of *Atherina* Species in Transmitting some Bacterial Diseases to Human**. Journal of American Science 2011;7(1):174-185]. (ISSN: 1545-1003). http://www.jofamericanscience.org		
28	**Quality of Life of School Age Thalassemic Children at Zagazig City** **Amal M El Dakhakhny[*1], Mervat A Hesham[2], Samah E Mohamed[3], Fawzia N Mohammad[4]** Pediatric Nursing Dewpt., Faculty of Nursing[1], Faculty of Medicine[2], Pediatric Nursing Dept.[3], Pediatric Nursing Dept.[4]- Zagazig University , Zagazig , Egypt dr_amal2001@yahoo.com[*] **Abstract:** Background: The assessment of quality of life in children, especially in those with chronic illness such as Thalassaemia, is particularly important. It differs from other forms of medical assessment in that it focuses on the individuals' own views of their well-being and other aspects of life, giving a more holistic view of well-being. The aim of the present study was to: assess the quality of life of school-age children with Thalassemia at Zagazig City. Subjects And Methods: A descriptive study was conducted on a sample of 100 school-age thalassemic children at out-patient Hematology clinic at Zagazig University Hospitals in Sharkia Governorate, Egypt. Two tools were used to collect the necessary data. The first was a structured interview questionnaire sheet including socio-demographic data of children and their parents as well as medical history. The second tool was a standardized tool (the Pediatric Quality of Life Inventory ™ Version 4.0). Results: The results of the present study revealed that the quality of life of school-age children with Thalassemia Major was affected. There was a significant association between the total quality of life and compliance with blood transfusion in both child and parent report. In addition, there was a significant association between the total quality of life and regular iron chelation therapy. Concluosion: Thalassaemia has a negative impact on perceived physical, emotional, social and school functioning in thalassaemia patients. Recommendations: Suitable programs aiming to increase children's adherence to the treatment regimen should be provided to increase psychosocial support. [Amal M El Dakhakhny, Mervat A Hesham, Samah E Mohamed, Fawzia N Mohammad. **Quality of Life of School Age Thalassemic Children at Zagazig City**. Journal of American Science 2011;7(1):186-197]. (ISSN: 1545-1003). http://www.jofamericanscience.org. **Key words:** Quality of life, thalassemia major, school-age	Full Text	28
29	***Saccharomyces cerevisiae* and Probiotic Bacteria Potentially Inhibit Fumonisin B_1 Production in Vitro and in Vivo** **Soheir Ahmed Al-Masri[1], Soha.M.S.El- Safty[2], Somaia A. Nada†[3] and Hassan A. Amra[4]** [1]Collage of Food Scines & Agriculture, King Saud University, Riyadh , Saudi Arabia , [2]Nutrition & food sciences, Home Economics Dept, Faculty of Education ,Suez Canal University, Ismailia. [3]Pharmacology Dept. and [4]Food Toxicology and Contaminant Dept. National Research Centre, Dokki, Cairo, Egypt somaianada@yahoo.com	Full Text	29

	Abstract: The objective of the present study was to evaluate the efficacy of probiotic bacteria: *Lactobacillus rhamnosus* GG (LGG), *Lactobacillus rhamnosus* (LC705) and *Saccharomyces cerevisiae* (*S.cerevisiae*) to inhibit *Fusarium moniliform* (*F. moniliform*) growth *in vitro* and to eliminate fumonisin B_1 from body of mature rat *in vivo*. *S.cerevisiae*, LGG and LC 705 potentially inhibited *F. moniliform* growth and fumonisin B1 production in YES liquid media. The biologically active microorganisms (S.cerevisiae, LGG & LC705) had no toxic effects in rats when orally administered single doses of *S.cerevisiae* (10^{11} CFU ml^{-1}) and LGG & LC705 (10^9 CFU ml^{-1}). Moreover, daily treatments for 15 days with the three microorganisms in saline concomitant with FB1 in corn oil (5 mg/ml FB1), produced *by F. moniliform*, exhibited significant reduction in serum ALT, AST, GGT, creatinine, and BUN compared with the positive control group *(F. moniliform*). Blood glutathione (GSH) level significantly increased ($P< 0.05$) in groups treated with single-treatment of *S.cerevisiae*, LGG & LC705 or with fumonisin B_1 containing media. However, fumonisin B_1 - treatment severely depleted GSH level than other treatments. The best results found in *S.cerevisiae* > LGG > LC705 -YES media containing fumonisin B_1. The tested microorganisms are safely to use as food additives or preservative due to their antioxidant activity. Our study needs further continuation in this respect. [Soheir Ahmed Al-Masri, Soha.M.S.El- Safty, Somaia A. Nada and Hassan A. Amra. ***Saccharomyces cerevisiae* and Probiotic Bacteria Potentially Inhibit Fumonisin B_1 Production in Vitro and in Vivo**. Journal of American Science 2011;7(1):198-205]. (ISSN: 1545-1003). http://www.jofamericanscience.org. **Keywords:** *Saccharomyces cerevisiae*, Probiotic bacteria, Fumonisin B1, *Fusarium moniliform*, rat, ALT, AST, GGT, creatinine, BUN and GSH		
30	**Correlation between Caregivers' Burnout and Elderly Psychological Abuse** **Fatma Mahmoud Mohammed Elemary[*1], Hanan Aboelgamelen Ebrahim Essa[2] and Hanaa Hamdi Aly[3]** [1]Psychiatric& Mental Health Nursing Department, Faculty of Nursing, Ain Shams University. Cairo, Egypt [2]Community Health Nursing Department, Faculty of Nursing, Tanta University. Tanta, Egypt [3]Psychiatric & Mental Health Nursing Department, Faculty of Nursing, Zagazig University, Zagazig, Egypt ya7ya_13@yahoo.com* **Abstract:** Psychological abuse of elders is a growing but hidden problem and is often under reported. Aim: this study aims to investigate the correlation between caregivers' burnout and elderly psychological abuse. Design: A descriptive correlational research design was utilized to conduct this study. Sample :It included 150 older person residing Dar El-Deiafaa, Dar El-Salam and Dar El-Zahraa for disabled and elderly people and 50 of caregivers (nurses& elderly sitters) ,who are working in these settings. Tools of data collection: include,1) socio-demographic data sheet concerned with caregivers' personal characteristics,2) Burnout Inventory developed by Maslach (1981),it was modified and translated into Arabic by the researchers and 3) Elder Abuse Screening Instrument developed by Fulmer et al (2004), that was modified and translated into Arabic by the researchers. Results: the study results revealed that, 34% of the studied caregivers their ages ranged from 35 to 40 years, 62% were male,52% their education at secondary stage & only 8% had university degree. Majority of them 64% worked as elderly sitter and 36% were nurses. 62% were unsatisfied with their paid, and 38% were satisfied with their paid. 58% had experience less than 5 years in their working with the elders, but 6% only had experience more than 10 years. Conclusion: There are strong positive associations between levels of caregivers' burnout and levels of elders' psychological abuse. Recommendations: I t is recommended that media coverage of abuse in elders homes has made the public knowledgeable about-and outraged against-abusive treatment in those settings, providing education, appropriate training and counseling for the caregivers to find solutions for their problems and the problems of the elderly and about the risk factors for abuse. [**Fatma Mahmoud Mohammed Elemary, Hanan Aboelgamelen Ebrahim Essa and Hanaa Hamdi Aly. Correlation between Caregivers' Burnout and Elderly Psychological Abuse**. Journal of American Science 2011;7(1):206-214]. (ISSN: 1545-1003). http://www.jofamericanscience.org	Full Text	30
31	**Synthesis and structure-activity relationship of new cephalosporins modified at C-7 and C-4**	Full Text	31

	H. M. Hassan*; S. A. Shedid; M. F. Badie and R. M. Eisawy Chemistry Department, Faculty of Science, Al-Azhar University, Cairo, Egypt hassanomar61@gmail.com **Abstract:** The synthesis and antimicrobial activity of a series of cefaclor derivatives bearing phthalyl or tosylaminoacyl or dipeptidyl moieties attached to the α-amino group of the 7-phenylglycinamido acyl unit, or amino acid residues and their corresponding methyl esters linked to the carbonyl group on C-4 are described. Some compounds of this series were found to possess high activity against pseudomonas aeruginosa and other Gram-negative bacteria. [H. M. Hassan; S. A. Shedid; M. F. Badie and R. M. Eisawy. **Synthesis and structure-activity relationship of new cephalosporins modified at C-7 and C-4**. Journal of American Science 2011;7(1):215-221]. (ISSN: 1545-1003). http://www.jofamericanscience.org. **Keywords:** Cefaclor, amino acids, antimicrobial activity		
32	Interactive Compromise Stability of Multi-objective Nonlinear Programming problems **Kassem, M.[(1)*], El-Benna, A.[(1)], and El-Badry, N.[(2)]** [(1)] Mathematics department, Faculty of Science, Tanta University [(2)] Mathematics department, Faculty of Science, Damietta Branch, Mansoura University **Abstract:** This paper presents a solution method for multi-objective nonlinear programming (MONLP) problems and stability of this solution. The method, offers a practical solution to MONLP problems by deriving the compromise weights and combining judgment with an automatic optimization technique in fuzzy decision making. This is achieved by using the method and algorithm of compromise programming and the method of compromise weights, and we obtain the stability for the solution in each step of the algorithm. A numerical example illustrates various aspects of the results developed in this paper. A maple procedure for this algorithm is introduced. [Kassem, M., El-Benna, A., and El-Badry, N., Interactive Compromise Stability of Multi-objective Nonlinear Programming problems. Journal of American Science 2011;7(1):222-229]. (ISSN: 1545-1003). http://www.jofamericanscience.org. **Keywords:** MONLP; Stability; Interactive decision making; Compromise weights; Membership functions.	Full Text	32
33	**Management of Recurrent Pterygia** **Ahmed A Zaki , Sherif Emerah , Mohamed Ramzy, Hany M Labib,** **Cornea and ocular surface unit, Research institute of ophthalmology, Cairo, Egypt** **Abstract: PURPOSE**: The objective of this study was to evaluate the postoperative outcomes of different surgical techniques with adjunctive therapy for the management of recurrent pterygia. **MATERIALS and METHODS:** Twenty eyes of twenty patients (7 females and 13 males, mean age 42.3 +/- 9.6 years) operated on for recurrent pterygia at the Research Institute of Ophthalmology, were recruited in this study. Patients were randomized into two groups: In group1, ten eyes of ten patients were done with conjunctival autograft and in group 2, ten eyes of ten patients were done with limbal conjunctival autografting. All eyes received intraoperative mitomycin C 0.01% for 3 minutes applied to the bare sclera at the time of the operation. The site of application of mitomycin C was thoroughly irrigated with balanced salt solution. All eyes were followed up every month for 12 months. **RESULTS:** After a mean postoperative follow up of 12 months, only one eye had a recurrence after 4 months in the limbal conjunctival autogrft group and there were two eye with recurrence after 2 and 4 month in the conjunctival autografting group (p = 0.027). No severe side effects appeared during the follow up period. **CONCLUSION:** This study confirms the efficacy of adjunctive therapy in improving the success rate after recurrent pterygium surgical excision. There was no difference between the two surgical procedures in the two groups, we also found no serious complications from using a low concentration (0.01%) of mitomycine C which was effective also in prevention of recurrences.	Full Text	33

	[Ahmed A Zaki , Sherif Emerah , Mohamed Ramzy, Hany M Labib. **Management of Recurrent Pterygia**. Journal of American Science 2011;7(1):230-234]. (ISSN: 1545-1003). http://www.jofamericanscience.org. Keywords: Management; Recurrent; Pterygia		
34	**Prevalence, Risk Assessment and Impacts of Eye Diseases among School Children in Cairo, Egypt** **Essam A. El-Moselhy[*1]; Hosam S. Abo-Seif[2]; Eman S. Abd Allah[3] and Ahmed A. Ghandor[1]** Department of Community Medicine[1]; Department of Ophthalmology[2] Faculty of Medicine Al-Azhar University, Cairo, Egypt. Department of Community Health Nursing[3], Faculty of Nursing, Zagazig University, Zagazig, Egypt. **Abstract:** Introduction: Eye diseases represent an important public health problem in childhood. Objectives: The aim of this study was to define the prevalence of different types of eye diseases, to assess risk of these diseases, and to determine the disease impacts on scholastic achievement of school students in Cairo, Egypt. Research design: A cross-section, analytical study design was chosen to perform this study. Research setting: The study was conducted in Al-Marg region, east district of Cairo. Four, randomly selected, schools were the field of the present study in this region. These schools were two primary schools (one public and one private) and two preparatory schools (one public and one private). Subjects and methods: The total number of students was 2160. All the students were examined clinically; for each case with eye disease a control case was chosen. The cases and controls were interviewed. Results: The study showed that 28.2% of the students have eye diseases. The most common eye diseases were trachoma (9.3%), errors of refraction (7.1%) and allergic conjunctivitis (6.3%). All eye diseases were more common in public schools. The most important significant socioeconomic and health care behavioral risk factors for eye diseases were the low level of parental occupation (OR=4.79), no early consultation for eye diseases (OR=3.13) and never received eye examination (OR= 2.68). Also, the most important significant personal characteristic risk factors were previous eye diseases (OR=3.35), positive consanguinity of the parents (OR=2.67), sibling(s) with eye diseases (OR=2.19), last birth order child (OR=1.90) and male sex (OR=1.56). Further, age and/or sex were significant risk factors for specific eye diseases; trachoma, errors of refraction, allergic conjunctivitis and muco-purulent conjunctivitis. Also, 37.7% of the students with eye diseases had significant school absenteeism 3-4 days/month (P=0.01) and 21.8% of them had significant results of the first term exam <50.0% (P=0.00). Conclusions: Eye diseases are prevalent among school students, especially in public schools in Cairo, Egypt. Many of the risk factors of eye diseases can be manipulated. So, these diseases and its negative impacts can be prevented. Recommendation: Improving students' and environment's hygiene, health education, regular eye screening and treatment of students as regard eye diseases in Egypt are an important essentiality. Also, eye health component of school health services should be integrated in school health program, and this should be integrated in medical and nursing curriculums. Lastly, further studies on large numbers of students in different rural and urban areas in Egypt are recommended. [**Essam A. El-Moselhy; Hosam S. Abo-Seif; Eman S. Abd Allah and Ahmed A. Ghandor. Prevalence, Risk Assessment and Impacts of Eye Diseases among School Children in Cairo, Egypt**. Journal of American Science 2011;7(1):235-246]. (ISSN: 1545-1003). http://www.jofamericanscience.org. Key words: Eye Diseases, School Children, Impacts	Full Text	34
35	**The Effect of Tacit Knowledge Characteristics on Tacit Knowledge Transfer: An Empirical Study within Egyptian Industry** **Mamdouh Refaiy** Associate Professor in Business Administration Business Administration Department, Faculty of Commerce, Ain Shams University, Cairo, Egypt. Mamdouh_Refaiy_17858@Hotmail.com **Abstract:** The purpose of this research paper is to examine the effect of tacit knowledge characteristics TKC on success factors to tacit knowledge transfer SFTKT from external sources such as suppliers, buyers, universities, and competitors to the recipient of knowledge. This research paper was based on questionnaire survey of Egyptian Industry Sector (75 companies) to investigate the range of attitude and	Full Text	35

	their ability to transfer both organisational and technological knowledge. The questionnaire was carried out by two ways; online, and the great majority via interviews questionnaire. In addition to, the empirical evidence collected from the survey confirms that the urgent need to continuous tacit knowledge transfer process in order to achieve a competitive advantage and sustainability. Additional, results suggest a strong positive effect of tacit knowledge characteristics on success factors to tacit knowledge transfer. As well as**,** empirical study involved the study of the tacit knowledge and classifying it into organisational and technological knowledge depends largely upon functional perspective. This was due to the user diversity**.** [**Mamdouh Refaiy. The Effect of Tacit Knowledge Characteristics on Tacit Knowledge Transfer: An Empirical Study within Egyptian Industry**. Journal of American Science 2011;7(1):247-263]. (ISSN: 1545-1003). http://www.jofamericanscience.org. **Key words:** tacit knowledge, tacit characteristics, organisational knowledge, technological knowledge, transfer factors, transfer barriers, Egyptian Industries Union		
36	**Evaluation of an experimental zinc phosphate cement powder** **Safwat EM[1], Saniour SH[2], Zaki DY [1] ,El-Batran MM[3], Mousa IM[2]** [1]Restorative and Dental Material Research Department, National Research Centre. Cairo, Egypt [2]Biomaterials Department. Faculty of Oral and Dental Medicine. Cairo University. [3]Basic Dental Science Department. National Research Centre. Cairo, Egypt. **Corresponding author**: Engie_safwat@hotmail.com Abstract: The aim of this study was to evaluate the properties of an experimentally prepared zinc phosphate cement powder. The working time, setting time, film thickness, compressive strength and solubility were tested for the experimental cement powder and compared with one of the commercially available zinc phosphate cement. Testing was done according to the ANSI/ADA specification No. (8) for zinc phosphate cement and No. (96) for dental water-based cements. Results revealed that the experimental cement produced working time, setting time, film thickness and solubility comparable with that specified by the ADA specification No. (8) and (96), and with that of the commercial cement, however the compressive strength (42.09 MPa) was significantly lower than that specified by the ADA No.(96) (70 MPa) but was not significantly different than that of the commercial cement (49.6 MPa). [Safwat EM, Saniour SH, Zaki DY, El-Batran MM, Mousa IM. **Evaluation of an experimental zinc phosphate cement powder**. Journal of American Science 2011;7(1):264-268]. (ISSN: 1545-1003). http://www.jofamericanscience.org. **Key words:** zinc phosphate cement, ANSI/ADA specification No.(8) and No.(96), working time, setting time, film thickness, compressive strength, solubility, disintegration	Full Text	36
37	**Assessment of Egyptian buffaloes crossing with Pakistani and Italian buffaloes for some production traits** Fooda, T. A.; Elbeltagi , A. R.; Laila R. Hassan and SetEl-habaeib S. Awad Animal Production Research Institute-Buffalo Breeding Research Department- Dooki- Giza – Egypt Tarek_Fooda@yahoo.com; Ahmed_elbeltagi@yahoo.com; lailarashad@hotmail.com; dr_habaeb@yahoo.com **Abstract:** Egyptian buffaloes are considered one of the most important dual purpose farm animals that represent 44% of dairy animals in Egypt. In 1980, the Animal Production Research Institute (APRI) imported 93 Pakistani semen straws for crossbreeding to improve milk productivities. In 2003, Ministry of Agriculture (MoA) allowed the commercial importation of Italian buffalo semen, which spread in large scale buffalo farms. The study aims to evaluate the Egyptian buffalo crosses with both Pakistani and Italian buffaloes for some productive traits to assess the crossing trials. For the first trial of the study, 180 records (85 pure Egyptian buffaloes (E), 22 record ½Egyptian (E)½ Pakistani (Pa) buffaloes and 52 record ¾E ¼Pa buffaloes and 21 record 7/8E 1/8Pa) through the period from 1980 to 1998 were used for the evaluation of Egyptian (E) Pakistani (PA) crossbred. Data for the second trial, concerned with the evaluation of the Egyptian (E) Italian (I) crosses, was collected from two private farms. A total 138 records; 64 record from Ganat Elreda farm (32 record E and 32 record ½E ½I) and 74 records from "United Group farm" (26 record E and 48 record ½E ½I buffaloes) was utilized. Utilized record covers the period from 2005 to 2009. Average for total milk yield was nearly the same for Egyptian and its cross with Pakistani buffaloes. In trial 1, Milk yield generally tended to increase with the advancement of parities till	Full Text	37

	the ≥7 parity**.** Egyptian buffaloes showed the highest values for all growth traits measures. In trial 2, significant difference in milk productivity between the Egyptian and its Italian crossbred, which was significantly higher ($P \leq 0.001$) in farm 2 than it is in farm 1 ($P \leq 0.01$), was observed. The same trend in difference was detected for the parity effect**.** Italian crosses showed higher least square means (LSM) estimates for total milk yield (TMY) than the Egyptian buffaloes, which also increase with the advancement of the parity, in the two farms. LSM data reveal increase of 27 and 15% in 1/2E1/2I crossbred milk production than the Egyptian in farm 1 and farm 2, respectively. Difference between the highest and lowest breeding value (BV) in the Egyptian population is larger than it is in the crossbred population. More studies are recommended for the assessment of productive, reproductive and genetic diversity of crossbred populations before the enhancement of crossbreeding activities on national level. [Fooda, T. A.; Elbeltagi, A. R.; Laila R. Hassan and SetEl-habaeib S. Awad. **Assessment of Egyptian buffaloes crossing with Pakistani and Italian buffaloes for some production traits**. Journal of American Science 2011;7(1):269-276]. (ISSN: 1545-1003). http://www.jofamericanscience.org. **Keywords:** Egyptian, Pakistani and Italian buffaloes, crossing, production traits, breeding value		
38	**Effect of Early versus Late removal of Urinary Catheter on Urinary Outcome after Hysterectomy** **Nahed F., Khedr.** Maternity and Gynecology Nursing, Faculty of Nursing,- Mansoura University **Abstract:** Aim of the study: this study aims to explore the effect of early versus late removal of urinary catheter on urinary outcome after hysterectomy. Setting:_This study was conducted in the gynecology department of Mansoura University Hospital. Study Design: quasi experimental design. Sample Type:- purposive sample. The study comprised of 100 gynecologic women, they were chosen according to the following criteria:-Complained from symptoms of uterine prolapse, undergoing hysterectomy, their age ranged from 40 ->60 years old and free from any other gynecological problems. They were categorized into two groups: 1) early group, had early removal of urinary catheter 12 – 24 hours after surgery. 2) late group had late removal of urinary catheter after surgery by 48 – 72 hr,s. Results: Urinary symptoms " retention of urine, frequency, burning micturation and UTI were significantly higher in late urinary catheter elimination group as compared to early removal group . Conclusion: Short duration of postoperative catheterization “12-24" hour's is preferred than long duration in which it lead to less urinary problems. Also age of women, degree and duration of uterine prolapse don't play a major role in development of post catheter removal urinary symptoms. Pre existing of postoperative UTI had a main role in the development of these symptoms. Thus it was recommended that ideal time of removal of urinary catheter is from 12-24 hour hysterectomy. [Nahed F., Khedr. **Effect of Early versus Late removal of Urinary Catheter on Urinary Outcome after Hysterectomy**. Journal of American Science 2011;7(1):277-281]. (ISSN: 1545-1003). http://www.jofamericanscience.org. **Key Words:** Urinary Catheter, Urinary Outcome, hysterectomy, pyelonephritis, Postoperative	Full Text	38

39	**Effect of protein feeding system on the quality of milk and its resultant Domiati Cheese** **EL-Sheikh, M.M.; S.A.H. Abo EL-Nor; Nadia M. Shahein and N.S. Abd Rabou** Dairy Department, National Research Centre, Dokki, Cairo, Egypt **ns_abdrabou@hotmail.com** **Abstract:** The use of Sunflower meal (SFM) and Leucaena leaves (LL) as a source of 30% of protein requirements in the feeding system of dairy buffaloes and its effect on the yield and composition of milk as well as its resultant Domiati cheese was investigated. The yield of fresh cheese was determined and cheese was pickled in salted whey for 4 months. Samples were taken from milk and also from cheese monthly during storage and were analyzed for moisture, fat, lactose, acidity, amino acids and nitrogen fractions. Formol & Schilovich ripening indices and total volatile fatty acids contents of cheese were estimated as well as their organoleptic properties.Using of SFM and LL increased total solids, fat and total protein of milk. However, the mean values of ash content of milk were lower for SFM and LL treatments. LL milk of LL was the highest in the essential amino acids.Satisfactory of fresh cheese yield (32.12%) for LL treatment, which was higher than control (30.25%) and SFM treatment (30.12%).No significant differences were found among all treatments for the gross composition. Domiati cheese made with LL milk showed the highest total nitrogen and the lowest acidity at the end of ripening period SN/TN % was higher with LL during ripening than SMF and control, while TVFA was higher with control than LL and SFM treatments. Ripening indices FRI & SRI shows that the LL ranged the higher values, followed by that made with SFM and control treatments. The total evaluation scores of fresh cheese were almost the same for all treatments. However, Domiati cheese from LL higher scores than control and SFM at the end of storage period. It can be concluded that sunflower meal and Leucaena leaves can be use as a source of 30% of protein requirements in the feeding system of dairy buffaloes and the milk yielded from this buffaloes can be successfully used in the manufacture of more quality of Domiati cheese. [EL-Sheikh, M.M.; S.A.H. Abo EL-Nor; Nadia M. Shahein and N.S. Abd Rabou. **Effect of protein feeding system on the quality of milk and its resultant Domiati Cheese**. Journal of American Science 2011;7(1):282-290]. (ISSN: 1545-1003). http://www.jofamericanscience.org. **Key Words:** Domiati cheese system, Sunflower meal ,Leucaena leaves	Full Text	39
40	**Application of Alpha mapping (α-mapping) of SP well-log Image, to obtain lithology and Correlate to evaluate the Reserves of Shan4 Depression of Shahejie formation China** Taiwo Olusoji Lawrence Department Petroleum and Natural Gas Engineering China Universityof Geosciences 430074 P.R China taiwosoji@gmail.com **Abstract:** Deducing geological parameters using SP curves is a very tedious, expensive, and error prone process such as obtaining formation water resistivity and the measurement of small negligible voltage potentials, mud filtrate resistivity and shale volume. This is due to the fact that there are many complex dependent variables surrounding data acquisition using Statistical method of data collection in an SP log image, these variables includes: borehole invasion, shale content, Bed resistivity and the ratio of salt water mud(R_{mf}) and fresh water mud (R_w). We have used Alpha (**α**) mapping method of SP-Log considering the shale content of the formation and the maximum possible deflection of Sp that a thick shale free porous and permeable formation can have at a given ratio of R_{mf} and R_w to obtain the lithology of Shan4 depression as well as limit error to the bearest minimum at a low cost of acquiring the petrophysical parameters. Based on the Structure map Shan4 depression in shahejie formation is composed of a complex depositional system of a prograded elongated delta, beach and bar formed under lower current energy of a shore-shallow lake. Hydrocarbon trap is created by an anticline pool separated by numerous oil layered complex faults, Oil and seeps in the depression are found in Tertiary sandstone reservoirs as well as underlying basement located at an approximate depth of 2020m below sea level (-2020m), including the Jurassic sandstone reservoirs and the carboniferous –Permian and Ordovician weathered zone.	Full Text	40

	[Taiwo Olusoji Lawrence. **Application of Alpha mapping (α-mapping) of SP well-log Image, to obtain lithology and Correlate to evaluate the Reserves of Shan4 Depression of Shahejie formation China**. Journal of American Science 2011;7(1):291-299]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** SP well-log; Shahejie formation; lithology; correlate		
41	**Simple Novel Spectrophotometric and Spectrofluorimetric Methods for Determination of Some Anti-hypertensive Drugs** **M. Farouk[1], O. Abd EL-Aziz`[1*], A. Hemdan[b], M. Shehata[2]** [1]Analytical Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ain Shams University, African Union, Cairo, Egypt. [2]Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ahram Canadian University, 6th October, Egypt. dr_omarghonim@hotmail.com* **Abstract:** Accurate, precise and selective spectrophotometric and spectrofluorimetric methods were developed and subsequently validated for determination of Torasemide (I), Irbesartan (II) and Olmesartan medoxomil (III), where (I) could be determined in presence of its acidic-degradate as stability indicating method, utilizing derivative ratio spectrophotometry, also in human plasma it could be determined by spectrofluorimetric method, (II) could be determined in a binary mixture with Hydrochlorothiazide (HCTZ) by simultaneous determination, utilizing ratio subtraction and spectrofluorimetric techniques, while (III) could be determined in presence of its alkaline-degradate as stability indicating method, utilizing derivative ratio and pH-induced difference spectrophotometric technique, also in a binary mixture with Hydrochlorothiazide (HCTZ), it could be determined by simultaneous determination, using ratio subtraction and spectrofluorimetric methods. All the proposed novel methods were validated according to International Conference of Harmonization (ICH) guide lines and successfully applied to determine the mentioned studied drugs in pure form, in laboratory prepared mixtures and in pharmaceutical preparations. The obtained results were statistically compared to the reference methods of analysis [for I, II and III, respectively] and no significant difference were found. [M. Farouk, O. Abd EL-Aziz, A. Hemdan, M. Shehata. **Simple Novel Spectrophotometric and Spectrofluorimetric Methods for Determination of Some Anti-hypertensive Drugs**. Journal of American Science 2011;7(1):300-]. (ISSN: 1545-1003). http://www.jofamericanscience.org. **Keywords:** Torasemide, Irbesartan, Olmesartan medoxomil, Derivative Ratio, Ratio subtraction, Difference Spectrophotometry, Spectrofluorimetry, Stability Indicating and Simultaneous Determination Methods	Full Text	41
42	**Repair Welding Restoration of the Screw Conveyor for Resin Extruder** **M. Amin[*1], S. M. Khafagy[2] and B. Zaghlool[1]** [1] CMRDI, Cairo, Egypt, [2] TIMS Cairo, Egypt morsy_abokhala@yahoo.com* **Abstract:** A screw conveyor was exposed to an extensive wear at the top and the side surfaces of the teeth. The microstructure of the base metal is martensitic structure. Welding procedure specification (WPS) and Process Qualification Record (PQR) were carefully performed using a scraped part from the screw conveyor. The preheating temperature of 300 to 400 °C was applied and the SMAW process was selected as selected as a welding process. Three types of electrodes were selected which mainly wear and corrosion resistance type. Using chromium Carbide electrodes resulted in a significant appearance of cracks at the weld surface that extended to the heat affected zone. However, Using martensitic electrodes resulted in a crack free weld metal with a significant improve of the wear resistance of the base metal. The effect of applying cushion layer between the base metal and hardfacing layer were studied using two kinds of covered electrodes. The hair cracks that observed using the hardfacing electrodes were greatly reduced using these cushion layers. The results were discussed on the basis of microstructure and the wear resistance of the base metal and the hardfacing layers. [M. Amin, S. M. Khafagy and B. Zaghlool. **Repair Welding Restoration of the Screw Conveyor for Resin Extruder**. Journal of American Science 2011;7(1):313-320]. (ISSN: 1545-1003).	Full Text	42

	http://www.jofamericanscience.org. **Keywords:** Welding; Restoration; Screw; Conveyor; Resin; Extruder		
43	**Assessment of Farmers Knowledge Regarding Innovation Management in Farming Cooperatives in Shoushtar Township, Iran** Ahmad Reza Ommani Assistant Professor Islamic Azad University Shoushtar Branch, Iran ommani75451@yahoo.com **Abstract:** The purpose of research is assessment of farmer's knowledge regarding innovation management in farming cooperatives in Shoushtar township of Khouzestan province, Iran. The method of research was correlative descriptive and causal relation. A random sample of Shoushtar township farmers of Khouzestan province, (n=105) were selected for participation in the study. According to results knowledge of farmers regarding management of innovation was moderate. Also regression showed that accessing to communication channel, level of education, income, crop yield, size of farm, social participation, level of participation in extension classes may well explain for 53% (R^2=0.534) changes in knowledge of farmers regarding management of innovation. [Ahmad Reza Ommani. **Assessment of Farmers Knowledge Regarding Innovation Management in Farming Cooperatives in Shoushtar Township, Iran.** Journal of American Science 2011;7(1):321-324]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Innovation Management, Farmers, Shoushtar	Full Text	43
44	**Calculate effects of synergism and antagonism of nutrient elements: nitrogen, phosphorus, potassium and sodium in maize** Tayeb Saki Nejad Assistant Professor Department of Agronomy Physiology, Islamic Azad University, Ahvaz branch * saki1971@iauahvaz.ac.irCorresponding Arthur: Tayebsaki1350@yahoo.com **Abstract:** Research projects in three consecutive years in 1999-2000 &2000-2001 and 2001-2002 years. Research Station - Research Azad University of Ahvaz were performed every three years in corn research using factorial experiment with a randomized complete block design with base 4 replications and two water stress factor with four levels as the first factor (**I_0** : Full irrigation point of FC, control, without water stress, **I_1**: 75% of the amount of irrigation treatments I_0, mild stress, **I_2**: 50% of the amount of irrigation treatments I_0, severe stress, I_3: 25% of the amount of irrigation treatment I_0, very severe stress and point of PWP) , period of growth with three levels as the second factor (V_1: vegetative period (until the emergence of the first deployment of plant double ring) V_2: reproductive period, V_3: the grain filling period in 3 years (1999-2000 &2000-2001 and 2001-2002) (Research Station, Islamic Azad University of Ahvaz 3 km south of Ahvaz city was designed and executed. Fertilizer amounts given in the first and second year experiment (1999-2000 &2000-2001) the same (N_{180} P_{70} K_0) was the third year of experiment (2001-2002) 20 percent of the amount of nitrogen and phosphorus fertilizers (N_{216} P_{84}) and the amount of 50 kg ha potassium fertilizer (K_2 O) to determine whether increased nutrient concentrations in the environment of plant roots in the same levels of water stress, changes in the process of accumulation of these elements in plant leaves, or not? Test results gathering process cluster to compare nutrient nitrogen, phosphorus, potassium and sodium in Different levels of water stress showed that the process of absorption and accumulation of nitrogen and phosphorus, two elements as well as potassium and sodium exclusively with each other at 1% level were similar. And because this was similar to that imposed different levels of water stress accumulation amount of both nitrogen and phosphorus element in the plant decreased, but the same amount of respect, two elements of K^+ Plant showed an increasing trend Regression analysis of variance in nutrient interaction at different levels of water stress, nutrient interaction with nitrogen phosphorus level of 5%, sodium potassium, nitrogen and potassium at 1% level significant effects on the interaction of elements and showed sodium diet with phosphorus, potassium and sodium phosphate with nitrogen did not provide significant effects. P interaction with N elements with correlation coefficient, linear regression fit showed that with increasing accumulation of nitrogen, phosphorus accumulation also increased with exercise and stress levels decrease Nitrogen accumulation was. Phosphorus accumulation process also presented a significant decrease. fit linear regression interaction of sodium with potassium correlation coefficient	Full Text	44

	showed that whatever amount was increased accumulation of potassium, sodium accumulation process of adjustment and provide significant levels Severe water stress that was greater accumulation of K, the process of absorption and accumulation of sodium than the control treatments (water stress) and mild stress (treatments) can be reduced. Increasing the nitrogen element, additive effect on the accumulation process with correlation coefficient K said that the effects on the control treatment (no water stress) was more evident at different levels of water stress by reducing nitrogen absorption, accumulation of ions to a very moderate state control part of his indicate that if the absorption of nitrogen in different treatments of water stress was not reduced, ion accumulation in the treatments than values obtained was estimated. [Tayeb Saki Nejad. **Calculate effects of synergism and antagonism of nutrient elements: nitrogen, phosphorus, potassium and sodium in maize.** Journal of American Science 2011;7(1):325-333]. (ISSN: 1545-1003). http://www.jofamericanscience.org. **Key words**: synergism & antagonism, nutrient elements, maize		
45	**Organochlorine pesticides (OCPs) in Breast milk in Hong Kong-Review** Ningombam Linthoingambi Devi[1], Qi Shihua[1], Ishwar Chandra Yadav[2] [1.]State key Laboratory of Biogeology and Environmental Geology, School of Environmental Studies, China University of Geosciences, 388, Lumo road, Wuhan 430074, China. nter of Advanced Study in Botany, Banaras Hindu University, Varanasi-225001, Uttarpradesh, India nldevi.cug@gmail.com **Abstract:** Organochlorine pesticides (OCPs) contaminant in human breast milk research is an environmental indicator. Because, diet is a major factor that influences breast milk levels of persistent organic pollutants, with patterns in fish consumption playing a particularly significant role. In this paper review available data on levels of organochlorine pesticides (OCPs), polychlorinated dibenzodioxins (PCDDs) in breast milk of Hong Kong. After reviewing all available data demonstrated that organochlorine pesticides consumption in Hong Kong is decreasing according to time trend. [Ningombam Linthoingambi Devi, Qi Shihua, Ishwar Chandra Yadav. **Organochlorine pesticides (OCPs) in Breast milk in Hong Kong-Review**. Journal of American Science 2011;7(1):334-340]. (ISSN: 1545-1003). http://www.americanscience.org. **Key Words**: Organochlorine pesticides; Human milk; Hong Kong	Full Text	45
46	**Customer Complaints Management: Concepts and Applications** Mohammad Taleghani Department of Management, Islamic Azad University, Rasht Branch, Iran Taleghani@iaurasht.ac.ir **ABSTRACT -** In this paper, Customer Complaints Management (CCM) and its associated key challenges were studied as essentials for achieving customer retention and loyalty. Some models illustrating the process of CCM were also demonstrated and discussed. A complaint intensity framework is presented, in which the joint distribution of complaint intensity and outcome satisfaction scores are conceptualized in four resulting quadrants with each quadrant suggesting a different CCM strategy. In empowering CCM, suggestions are proposed and Return on Complaint Management (ROCM) is described as a performance indicator for complaint management profitability. Major findings indicate that effective complaints management requires a cultural change in organization's atmosphere, as well as a systematic approach; different levels should be considered in complaints management; employees participating in teams play an important role in succeeding the complaints handling processes; and CCM empowerment should include strategy, processes, and analysis. [Customer Complaints Management: Concepts and Applications. **Customer Complaints Management: Concepts and Applications**. Journal of American Science 2011;7(1):341-347]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Customer, Satisfaction, Complaints, Management, Handling, Empowerment	Full Text	46
47	**Characterization of ZnS Quantum dot (q-dot) by Ultraviolet Visible (UV-VIS) Absorption Spectrum Studies & Comparison with CuO Nanocrystal** **Mamun Mohanty[1], Aurobinda Acharya[2], Bairagicharan Panda[3], Selvaraju Balamurgan[4], Subhendu**	Full Text	47

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ABSTRACT: Ultrasize ZnS quantum dots have been synthesized with (3-Mercatopropyl) trimethoxysilane as the capping agent by the all-aqueous procedure. The size of quantum dot by this method is in the range 4 nm to 10 nm. These quantum dots have been characterized by UV-Visible absorption spectrum. The absorption spectrum of synthesized quantum dots indicate a blue shift with decrease of size of quantum dot. Further UV-Visible absorption spectrum of quantum dot has been compared with that CuO nanocrystal.
[Mamun Mohanty, Aurobinda Acharya, Bairagicharan Panda, Selvaraju Balamurgan, Subhendu Pattnaik, Gourisankar Roy. **Characterization of ZnS Quantum dot (q-dot) by Ultraviolet Visible (UV-VIS) Absorption Spectrum Studies & Comparison with CuO Nanocrystal**. Journal of American Science 2011;7(1):348-351]. (ISSN: 1545-1003). http://www.americanscience.org.
Keywords: Quantum dots, UV-Visible spectrum, Blue shift

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Therapeutic and Protective Effects of Biphenyl Dimethyl Dicarboxylate (DDB) and Silymarin in Human Infected with HCV and in Carbon Tetrachloride Induced Hepatitis in Rats

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Abstract: This investigation aimed to evaluate the therapeutic activity of pure and commercial products of Biphenyl Dimethyl Dicarboxylate (DDB) and Silymarin in humans suffering from HCV and therapeutic and protective effects of Carbon tetrachloride (CCL4) induced liver damage in rats. *Humans* were divided into two groups: Group I: Normal controls (N=20), and group II: Patients suffering from chronic HCV infection; which were subdivided into two subgroups: A, ten patients received Silymarin 140 mg twice daily for one month and B, twenty patients received DDB 10 pilules (15 mg) twice daily for one month. Samples from control and treated groups were collected and obtained serum was analyzed for Aspartate aminotransaminase (AST), Alanine aminotransaminase (ALT), Alkaline phosphatase (ALP or Alk.ph.), Gamma Glutamic transaminase (GGT) and Serum bilirubin (total and direct). In addition, the effect of DDB or Silymarin administration on the mentioned biochemical parameters was measured. Other experiment was conducted in which rats were divided into nine groups, each group comprising of six rats. All rats except the control group were subjected to administration of Silymarin or DDB in pure and commercial products, before and after treatment with CCL4. All serum samples of rats were subjected to liver function tests including: (AST), (ALT), (ALP.) and serum bilirubin as well as kidney functions tests including: blood urea and serum creatinine. Histopathological examination of liver tissues was also performed. The results revealed that DDB improved liver functions in patients suffering from HCV infection, while Silymarin showed insignificant alteration for the same parameters. The raw and commercial products of Silymarin or DDB were significantly improved liver, kidney functions and the histopathological changes after induction of CCL4 toxic hepatitis in rats. Administration of DDB (commercial) for one month to patients suffering from chronic viral hepatitis resulted in a rapid decrease in serum transaminases, especially ALT. Treatment of rats by pure and commercial DDB for 7 days showed improvement in acute hepatocellular necrosis or hepatitis-associated hepatocellular damage caused by

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	carbon tetrachloride. Administration of commercial Silymarin for one month was largely ineffective in patients suffering from viral hepatitis. The results of 7 days treatment by pure and commercial products of Silymarin in rats showed protection of liver tissue. Silymarin has an antioxidant effect. In rats Silymarin increased the level of total protein which indicates hepatoprotective activity as results of accelerate of regeneration process and production of liver cells. Obtained histopathological study confirmed the results of biochemical studies. It is concluded that a superiority and efficacy of DDB over Silymarin in normalizing the liver enzymes and serum bilirubin (total and direct) levels were achieved after treatment of humans suffering from HCV. [Wassfy A. A., Ellaithy H. M., Hamza Y. E., Arbid M. S., Osman A.H., and Kandil S. M. **Therapeutic and Protective Effects of Biphenyl Dimethyl Dicarboxylate (DDB) and Silymarin in Human Infected with HCV and in Carbon Tetrachloride Induced Hepatitis in Rats**. Journal of American Science 2011;7(1):352-364]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** DDB, Silymarin, humans, HCV, Rats, CCL4, hepatotoxicity		
49	**Design and Manufacturing of Parabolic Trough Solar Collector System for a Developing Country Pakistan** Nusrat Kamal Raja [1], M. Shahid Khalil [2], Syed Athar Masood [3], Muhammad Shaheen[4] [1,2] Dept of Mechanical Engineering, UET Taxila, Pakistan [3] Dept of Engineering Management, NUST College of E & ME, Rawalpindi Pakistan [4] Dept of Computer Science & Engg, UET Lahore, Pakistan [1] kamalraja62@yahoo.com, [2] shahid.khalil@uettaxila.edu.pk, [3] atharmasood2000@hotmail.com, [4] shaheen@uet.edu.pk **Abstract:** Pakistan's thirst for electric power has been constantly rising over the years because of population growth, increase in industrial activity and failure of other resources for producing enough energy to meet its growing energy demand, particularly in the remote areas where energy is most needed. Pakistan is basically an energy deficient society and now going towards extreme energy crisis. Moreover, with current demand growth at 8 % annually, Pakistan will have to add 4000 MW to its existing capacity by the year 2018. Pakistan is rich in renewable energy resources; particularly solar energy has a special relevance in Pakistan due to high availability of Sun radiations at an average rate of 4.5-6 kwh / m^2 / day. The purpose of this research is to reduce the cost of conventional power plant by focusing on simplifying the design of collector structure to achieve a high reflecting quality and tracking precision, using available cost effective components, minimizing field construction requirements, and by utilizing the advantages of design engineering and equipment specifications as per environmental impact at feasible locations in most remote and energy starved areas of Pakistan. Most of the area of Pakistan lies in sunny belt of the earth with the sun shine of 6 – 8.5 hours daily having the greatest amount of radiant energy more than 90% of solar radiation, which comes as direct radiation because of the limited cloud coverage and clear sunny weather is experienced 250 to 300 days a year. Different concentrating technologies have been developed or are currently under development for various applications. The Parabolic Trough Solar Collectors system will undoubtedly provide within next decade a significant contribution to efficient, economical, sustainable renewable and clean energy supply to developing countries with positive effect on environmental activities. The collector materials will be used considering conversion efficiency, abundance of the material, low cost structures, ease of application, expected lifetime, and the availability of space at the collection site. Available sites in Pakistan desert can theoretically cover the whole electricity demand of the country. A small configuration system like 25KW can lead to 100MW by scale up as sub unit of larger power plants. This will be the first step to fulfill the energy demand of Pakistan, which has become essential for our economic revival. [Nusrat Kamal Raja, M. Shahid Khalil, Syed Athar Masood, Muhammad Shaheen. **Design and Manufacturing of Parabolic Trough Solar Collector System for a Developing Country Pakistan.** Nusrat Kamal Raja, M. Shahid Khalil, S. Athar Masood, M. Shaheen. Design and Manufacturing of Parabolic Trough Solar Collector System for a Developing Country Pakistan. Journal of American Science 2011;7(1):365-372]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Species richness; beta-diversity; taxonomic diversity; forest	Full Text	49
50	**Influence Of Choline Chloride On Quality And Storability Of Peach Fruits Cv. Earligrande.**	Full Text	50

	Wahdan, M. T. * and Faten, H. M. Ismaeil ** *Hort. Dep. Fac. of Agric. Suez Chanel Univ. Wahdan2020@yahoo.com ** Agric. Botany. Dep. Fac. of Agric. Benha Univ. fatenismaeil@yahoo.com **ABSTRACT:** The effects of preharvest foliar application of Choline Chloride (CC) on fruit quality of "EarliGrande" peaches at harvest and during cold storage at 1ºC temperature was investigated. CC was sprayed at concentrations of 0, 500, 1000, 1500 and 2000 mg/L at 30 days preharvest time (DPH). Fruit weight was increased by 500, 1000 and 1500 mg/L CC. At the same concentrations SSC/TA ratio was increased while, fruit acidity was decreased. Sugar, phenol and vitamin C content tended to increase by CC at harvest time. The combination of CC treatments at 1000 and 500 or 1000 mg/L and cold storage at 1ºC resulted in a reduction of weight loss (%) in two seasons, respectively. CC in combination with storage resulted in higher fruit firmness, SSC, SSC/acidity and total sugar and a reduction in fruit acidity in both seasons. [Wahdan, M. T. and Faten, H. M. Ismaeil. **Influence Of Choline Chloride On Quality And Storability Of Peach Fruits Cv. Earligrande**. Journal of American Science 2011;7(1):373-381]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** peaches, EarliGrande, Choline Chloride, fruit quality, storability		
51	**The Preparation of Paddy Map by Digital Numbers of IRS images and GIS** Mohammadi Torkashvand A. Department of Horticulture, Agriculture Faculty, Islamic Azad University-Rasht Branch, Rasht, Iran Torkashvand@iaurasht.ac.ir, m.torkashvand54@yahoo.com **Abstract:** Preparing updated map of paddy is an important map in the management and region agricultural planning. In this research, surveying of paddy investigated using IRS Satellite images in the Roudbar region, Guilan, Iran. The mean and standard deviation of training and auxiliary pixels of paddy was calculated. Upper and lower limits of DN-olive orchards were distinguished by the adding standard deviation to mean or diminishing of that. After rounding the upper/lower limits of paddy spectrum reflexes, 22-25, 40-98 and 24-136 of spectrum reflexes limits had been considered for bands 1, 2 and 3 with paddy class. In each band, Paddy limits introduced to software and slicing method used to prepare paddy map. Final map of paddy obtained from crossing of these three maps. The paddy map has been crossed by training point map to calculate the accuracy of method. The results indicate that in classification of images with spectrum reflex statistics, more than 73% of training points had again paddy class in the paddy fields classified map. [Ali Mohammadi Torkashvand, The Preparation of Paddy Map by Digital Numbers of IRS images and GIS. Journal of American Science 2011;7(1):382-385]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Species richness; beta-diversity; taxonomic diversity; forest	Full Text	51
52	**Study of Some Chemical Pollutant Residues in Catfish at Sharkia Governorate, Egypt** **Salah El- Dien, W.M. and Hend, A. Mahmoud*** Animal Health Research Institute, Dept. of Food Hygiene, Zagazig Provincial Lab., Egypt *Pesticide Residue Dept., Central Pesticide Lab., Agricultural Research Center, Egypt. **ABSTRACT:** Thirty samples of African catfish (*Clarias gariepinus*) were collected from the markets in Sharkia Governorate for detection and determination of 13 organochlorine pesticides (αBHC, βBHC, γBHC, heptachlor, heptachlor epoxide, aldrin, dieldrin, endrin, γ chlordane, γ endosulfan, pp DDE, pp DDD and pp DDT), 5 organophosphorus pesticides (diazinon, chlorpyrifos, chlorpyrifos methyl, profenophos and disyston) and 11 polychlorinated biphenyls (PCBs) congeners (PCB28, PCB44, PCB70, PCB101, PCB105, PCB138, PCB152, PCB153, PCB180, PCB192, and PCB194). All the tested organochlorine pesticides were detected with the frequency ranged between 30% for αBHC and 76.66% for aldrin + dieldrin. Their mean concentrations varied from 1.9 ppb for aldrin to 122.2 ppb for γBHC. Meanwhile all the tested PCBs were detected except PCB105 with the frequency lies between 10% for PCB28 and 53.3% for PCB152, while; the mean concentrations varied from 3.0 to 89.16 ppb for PCB194 and PCB152 respectively. All the estimated organochlorine pesticides and PCBs were below the	Full Text	52

	permissible limits in all the examined samples. Meanwhile, the tested organiphosphorus compounds were not detected in all the examined samples. The relatively high frequency and levels of organochlorine pesticides and PCBs may be explained by the nature of catfish habits and feeding as exhibited in this study. [*Salah El- Dien, W.M. and Hend, A. Mahmoud.* **Study of Some Chemical Pollutant Residues in Catfish at Sharkia Governorate, Egypt**. Journal of American Science 2011;7(1):386-393]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Chemical Pollutant; Residue; Catfish; Sharkia; Governorate; Egypt		
53	**Surface Morphology of the Tongue of the Hoopoe (*Upupa Epops*)** **Neveen E.R. El-Bakary** Department of Zoology, Faculty of Science, Damietta Branch, Mansoura University, New Damietta, Egypt. elbakaryneveen@yahoo.com **Abstract:** The tongue of birds fills the oral cavity and has a beak- like shape. The hoopoe's beak is long, slender and slightly down curved, however, the hoopoe's tongue is reduced in the buccal cavity. Several studies have shown morphological differences among the tongue of bird species. The aims of this study was to examine the dorsal lingual surface of hoopoe's tongue using scanning electron microscopy and to compare the present results with those reported in other avian species. The Hoopoe's tongue occupy 2/3 length of the beak. The morphological features observed in the lingual surface are follows; the epithelium of the apex is thickly keratinized, large conical papillae are located at the border between lingual apex and body, small conical papillae are located between lingual body and root and numerous lingual glands are located in the anterior part of the lingual body and in the clefts of the lingual root. The observations of the three dimensional structure of the subepithetial connective tissue revealed the presence of a system of laminae or smaller interconnected ridges, depending on the area of the tongue. We have indicated the possibility that the differences in the structures of the avian tongue related to the differences in the feeding habits. [Neveen E.R. El-Bakary. **Surface Morphology of the Tongue of the Hoopoe (*Upupa Epops*)**. Journal of American Science 2011;7(1):394-399]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** tongue, birds, hoopoe, scanning electron microscopy	Full Text	53
54	**Chronic Asthmatic Chest Troubles and Their Effects on Cognitive Functions, Psychosocial Behaviour and Academic Achievment among Children in Egypt** Samuel S*, Safwat M*, Morcos W**, Salem S**, El-Adly T*and Mohammed A. *Department of Paediatrics, Faculty of Medicine, Cairo University **Department of childhealth, National Research center **samarmsalem@hotmail.co.uk** **Abstract:** Chronic illness is clearly an important factor affecting psychosocial state of children and adolescents. This case-control study is an effort to clarify the effect of chronic asthmatic chest troubles as a chronic illness on the cognition and psychological aspects of such chronically ill children. This was a case control study conducted at the Chest Clinic of the Abou El-Reesh Children's Hospital, Cairo University. It included 23 children suffering from chronic asthmatic chest troubles (13 boys and 10 girls) with an age range of 6-15 years and a mean age of 9.6±2.67(± SD). Twenty three age and sex matched healthy children and living under the same socioeconomic conditions were taken as controls. The Arabic Version of the Revised Wechsler Intelligence Scale for Children (WISC-R) and Pediatric Symptom Checklist (PSCL) were used to assess the cognitive and psychosocial adjustment among children while the mid-year scores for Mathematics and Arabic language were used to evaluate the academic performance.Our results indicated that chronic asthmatic disease has a negative effect on cognitive abilities, psychosocial behavior and academic achievement of such children. [Samuel S, Safwat M, Morcos W, Salem S, El-Adly T and Mohammed A. **Chronic Asthmatic Chest Troubles and Their Effects on Cognitive Functions, Psychosocial Behaviour and Academic Achievment among Children in Egypt**. Journal of American Science 2011;7(1):400-406]. (ISSN: 1545-1003). http://www.americanscience.org.	Full Text	54

	Keywords: Children-chronic, asthma-congitive, function-psychosocial, behavior-academic, achievement		
55	**Insulin-mimetic activity of vanadium and zinc in diabetic experimental rats** ***Nabila, M. Rashwan and **Farida Abdullah Al-Firdous** [*]Home Economics Dept, Faculty of Education, Suez University, Ismaelia Egypt. **Department of Nutrition and Food Science , Home Economic , Collage ,Princess Nora Bent abdul – rahman -University, Riyadh, Saud Arabia **Abstract:** Forty-two adult male albino rats Sprague –Dawley strain were classified into normal control group and five diabetic rat groups which were control (+ve), drug, zinc , vanadium and zinc with vanadium. The diabetic control (+ve) group showed a significant increase in the values of glucose ,glucosalated hemoglobin ,serum alanine and aspartate amino transferase (ALT & AST), alkaline phosphatase (Alk-phos) enzymes, creatinine , urea ,cholesterol, triglyceride (TG), LDL-c, VLDL-c level , cholesterol/ HDL-c ,liver cholesterol, liver total lipid and liver malondialdehyde (MDA) but a significant decrease in final weight, weight gain, FER, insulin, hemoglobin (HB) , packed cell volume ,HDL-c ,liver glycogen, liver glutathione peroxidase (GPX)compared to normal control group. The diabetic rat groups which treated with drug, zinc, vanadium and zinc with vanadium showed a significant decrease in the values of serum glucose , glucosalated hemoglobin, ALT ,AST ,urea, serum cholesterol, triglyceride (TG), LDL-c, VLDL-c level , cholesterol/ HDL-c ,liver cholesterol, liver total lipid and liver malondialdehyde (MDA) but a significant increase in the values of final weight, weight gain percent , FER ,insulin ,packed cell volume (PCV) ,HDL-c ,liver glycogen and glutathione peroxidase (GPX) compared to control (+ve) group. [Nabila, M. Rashwan and Farida Abdullah Al-Firdous. **Insulin-mimetic activity of vanadium and zinc in diabetic experimental rats**. Journal of American Science 2011;7(1):407-416]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** vanadium, zinc, diabetes & rat	Full Text	55
56	**Economic crisis in Guilan textile industry** Seyed Ali Mirebrahimi ,Hamidreza Alipour Department of management,economic,collage of management,Islamic Azad University, Rasht Branch, Iran, drbehdad_66@yahoo.com **Abstract:** Today, industrial development is account as one of means and area for the economic development and improvement of the countries which some of the industrial courses that exist in any country due to relative advantages are account of high priority in industrial development area. texile industry, is account as the most important and oldest industry of the country and Guilan province. It can play a role as the main base of industry and mine sector if there is the required support from producers . But still it is not taken place a remarkable activities as developmental region planning in Guilan and it could not find a scientific and professional figure. So, the main goal of this article is identifying the variables and tensor factors in the Guilan texile industry and turning ways from current situation to modern developmental situation . This research had been attempted to study how turning out of the created crisis aiming to identify the crisis in texile industry and also allocating the optimal resources. The results indicated that the most important and significant problem of texile industry in Guilan is weak in management area . [Seyed Ali Mirebrahimi and Hamidreza Alipour. Economic crisis in Guilan textile industry. Journal of American Science 2011;7(1):417-421]. (ISSN: 1545-1003). http://www.americanscience.org. **keywords :** crisis, economy, texile industry, private sector, technology	Full Text	56
57	**Effects of Aldosterone Receptor Antagonist on Vascular Calcification and Bone Disorder in Streptozotocin-Induced Diabetic Rat** **Shadia A.E. Barakat [1], Nermine K.M. Saleh [1]*, Sahar S. Thabet [1], Hanan A. Saleh [2] and Abd El-Hamid A. Mohamed [1]** Physiology[1] and Histology [2]Departments, Faculty of Medicine, Ain Shams University, Cairo, Egypt	Full Text	57

	* nermine_saleh@yahoo.com **Abstract:** Background: Vascular calcification and bone disorders are increasingly recognized problems in patients with diabetes due to calcium dyshomeostasis is a major risk factor for cardiovascular morbidity and mortality. Diabetic osteoporosis seems to be dependent on qualitative and quantitative alterations of the bone, as well as microangiopathic complications of diabetes mellitus. Aim: We investigated calcium dyshomeostasis, and bone histological and metabolic abnormalities in Streptozotocin-induced Type 1 Diabetes Mellitus in rats. The possible role of the aldosterone receptor antagonist, spironolactone, in reversing these effects was assessed. Materials and Methods: Adult Female Wistar rats were divided into three groups: Control group, Streptozotocin-induced diabetic group (STZ-D), and Aldosterone-receptor antagonist-supplemented diabetic group (ARA-STZ). Diabetes was induced by a single intraperitoneal injection of streptozotocin, 40 mg/Kg BW. Spironolactone (aldosterone receptor antagonist) was given by oral gavage in a daily dose of 15 mg/kg BW for 4 weeks. At the end of the experiment, serum levels of calcium, phosphate, and alkaline phosphatase were evaluated. Histological examination of the tibia was performed, together with analysis of renal vascular calcification and Immunohistochemistry for inducible nitric oxide synthase (iNOS) in renal tissue specimens. Results: STZ-D rats showed normophosphatemia and significant hypercalcemia with significantly increased serum alkaline phosphatase compared to control group. Bone loss was also observed. Histological examination of the small renal blood vessels showed calcification in the walls, as well as, reduction in iNOS immunostaining. These metabolic and histological abnormalities in STZ-D rats were remarkably corrected by the administration of spironolactone. Conclusion: The current results underscore the important role of aldosterone in promoting vascular calcification and osteoporosis in diabetic rats and the potential role of aldosterone receptor antagonist, spironolactone, in correcting these clinical problems in diabetic rats. [Shadia A.E. Barakat, Nermine K.M. Saleh, Sahar S. Thabet, Hanan A. Saleh and Abd El-Hamid A. Mohamed. **Effects of Aldosterone Receptor Antagonist on Vascular Calcification and Bone Disorder in Streptozotocin-Induced Diabetic Rat.** Journal of American Science 2011;7(1):422-430]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words:** Ca^{2+} homeostasis, osteoporosis, vascular calcification, DM, Aldosterone.		
58	**The effects of peer education on health behaviors in girls with dysmenorrhea** Zahra Abedian[1], Maryam Kabirian[2], Seyed Reza Mazlom[3], Behroz Mahram[4], Mehrdad Jalalian[5,6] [1.] Faculty Member, Department of Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran [2.] MSc. Student in Midwifery, Department of Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran [3.] Faculty Member, Department of Medical & Surgical Nursing, Mashhad University of Medical Sciences, Mashhad, Iran [4.] Faculty Member, Mashhad Ferdowsi University, Mashhad, Iran [4.] Department of Community Health, faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selandor D.E., Malaysia [4.] Research Center of Iranian Blood Transfusion Organization, Khorasan Razavi Blood Center, Mashhad, Iran kabirianm1@mums.ac.ir **Abstract:** This study was conducted to compare the effect of peer-led VS health-provider-led self-care education on dysmenorrheic girls' knowledge, attitude, and menstrual symptoms of primary dysmenorrhea at dormitories of Ferdowsi University in Mashhad, Iran. In this randomized clinical trial, 165 girls between ages 19-25 who had experienced menstrual cramps three or more times during the last six months were randomly assigned to three groups (peer-led self-care education, health-provider-led self-care education, and control). A Menstrual Knowledge Questionnaire (MKQ), Menstrual Attitude Questionnaire (MAQ), and Menstrual Information Form were the main instruments in this study. Data were collected in the baseline menstrual period and one and two menstrual periods after intervention. One-way ANOVA and Kruskal-Wallis were used to analyze data by SPSS software. Menstrual Knowledge in the peer-led self-care education group increased 2.1 times and 2.5 times in the health-provider-led self-care education groups. Negative concepts of menstrual attitude decreased in the peer-led self-care education group (56.6	Full Text	58

	vs. 40.2, p=0.009) more than the health-provider-led self-care education group (56.9 vs. 48.3, p=0.035). There was no significant difference in the measure of decrease in pain score between interventional groups at both the first (p=0.988) and second (p=0.965) menstrual periods after intervention. These findings provide preliminary evidence that peer education can be effective health promotion in primary dysmenorrheic girls. **[**Zahra Abedian, Maryam Kabirian, Seyed Reza Mazlom, Behroz Mahram, Mehrdad Jalalian. **The effects of peer education on health behaviors in girls with dysmenorrheal.** Journal of American Science 2011;7(1):431-438]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** peer education; health behaviors; primary dysmenorrhea		
59	**Purification, Characterization and Antitumor Activity of L-asparaginase from Chicken liver** EL-Sayed , M. El-Sayed[1] , Sanaa T. El-Sayed[*2], Wafaa, G. Shousha[1], Abeer, N. Shehata[2] and Shimaa, S.Hanafy[2] [1]Biochemistry, Chemistry Department, Faculty of Science, Helwan University, Helwan, Egypt [2] Biochemistry Department, National Research Center, DoKKi, Giza, Egypt. santsayed@yahoo.com[*] **Abstract: Abstract:** The L-asparaginase (E.C.3.5.1.1) produced by chicken liver was isolated and characterized. Different purification steps (including ammonium sulphate fractionation followed by separation on Sephadex G-100 gel filtration and Sephadex G-200 gel filtration) were applied to crude filtrate to obtain a pure enzyme preparation. The enzyme was purified 128.5 ± 0.5 fold and showed a final specific activity of 158.11 ± 5.0 U/mg with a 17.1 ± 8.6 % yield. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of the purified enzyme revealed it was one peptide chain with M_r of 33 kDa while by gel filtration appears to be 36 kDa. The enzyme was very specific for L-asparagine and doesn't hydrolyze L-glutamine. A Lineweaver-Burk analysis showed a K_m value of 1.66 mM toward L-asparagine as substrate and V_{max} of 34.47 U. The enzyme showed maximum activity at pH 9.5 when incubated at 60°C for 20 min. The amino acids composition of the purified enzyme was also determined. Antitumor activity was investigated. The enzyme inhibited the growth of the two human cell lines including hepatocellular carcinoma (Hep-G2) and colon carcinoma (Hct-116) with IC_{50} value of 8.38µg/ml and 4.67µg/ml, respectively. While IC_{50} was greater than 10µg/ well for MCF7 (breast carcinoma) cell line. [EL-Sayed, M. El-Sayed, Sanaa T. El-Sayed, Wafaa, G. Shousha, Abeer, N. Shehata and Shimaa, S.Hanafy. **Purification, Characterization and Antitumor Activity of L-asparaginase from Chicken liver**. Journal of American Science 2011;7(1):439-449]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords**: Chicken liver- gel filtration-purification-amino acid composition- human cancer cell line-antitumor activity	Full Text	59
60	**Nursing Intervention Program for Early Detection and Prevention of Breast Cancer among Working Women** Nahla Ahmed Abd El-Aziz[*1], Fathia Ahmed Mersal[1] and Nadia Mohamed Taha[2] [1] Community Health Nursing, Faculty of Nursing, Ain Shams University, Cairo. Egypt [2]Medical Surgical Nursing, Faculty of Nursing Zagazig University, Zagazig. Egypt nahla_eassawy@yahoo.com [*] **Abstract:** Aim: of the study was to assess the impact of a nursing intervention program leading to health decisions for breast cancer screening among working women with the hypothesis that the intervention will improve women knowledge, modify their attitude, and empower them to take informed health decisions for breast cancer screening. Design: This quasi-experimental design Setting: was conducted in 2 pharmaceutical companies, 2 food processing industries, and a textile factory Sample: a convenience sample 520 women working previous settings, Tools: used for data collection included a self-administered assessment questionnaire assessing knowledge, a health beliefs assessment rating scale, an attitude rating scale, a breast self-examination observation checklist, and a mammography card. A nursing intervention program was designed by the researchers based on the results obtained from the study tools and findings of	Full Text	60

	similar research. Results: The mean age of studied women was 43.2 years, and 56.7% of them had secondary education. Only 5.4% of the women had satisfactory knowledge at the pretest. After program implementation, statistically significant improvements were revealed in women's knowledge about breast cancer and early detection methods, as well as in their related health beliefs and attitudes .Also,73.3% and 72.9% women successfully perform BSE at the post and follow-up phases ($p<0.001$). The practice of mammogram increased from 4.2% at the pre-intervention to 17.7% at the follow-up ($p> 0.001$). The highest practices were among women working in pharmaceutical companies, those with age 45 of older, and those with positive family history of breast cancer. Conclusion: Working women had deficient knowledge, and negative perceptions related to breast cancer and its early detection; their practice of breast self-examination and mammography was very low. The intervention program had a positive effect on women's knowledge, practice health beliefs and attitude. Recommendations: Continuous workplace educational health programs are recommended. With supportive health insurance. Further research studies with broader range of occupational setting are suggested. [Nahla Ahmed Abd El-Aziz, Fathia Ahmed Mersal and Nadia Mohamed Taha. **Nursing Intervention Program for Early Detection and Prevention of Breast Cancer among Working Women**. Journal of American Science 2011;7(1):450-459]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Nursing; Intervention; Breast Cancer; Women		
61	**_In Vitro_ Maturation of Camel Oocytes As Affected By Different Media during Breeding and Non-Breeding Seasons** A.E.B. Zeidan[1], M.A. El-Harairy[2], Sh.A. Gabr[3], M.A. Tag El-Dien[1], S. A. Abd El-Rahman[4] and A.M. Amer[1] [1]Animal Production Research Institute, Dokki, Giza, Egypt. [2]Department of Animal Production, Faculty of Agriculture, Mansoura University, Egypt. [3]Department of Animal Production, Faculty of Agriculture, Tanta University, Egypt. [4]Biology Department, Faculty of Science, Al-Mostansiriya University, Iraq. **Abstract:** A total number of 220 clinically healthy she-camel was used in this study. The age of these camels varied from 5 to 10 years and their weights were approximately 500-600 kg. Two experiments were carried out. The first experiment aimed to define the effect of different seasons of the year on follicular fluid components and ovarian activity either in the right or left ovary. The second experiment designed to define the effects of various maturation media (TCM 199, Ham's F-10, Basal and Hank's) on the *in vitro* maturation of camel oocytes during breeding and non-breeding seasons. In the first experiment, the obtained results showed that ovary weight and number of corpora lutea were significantly ($P < 0.05$) higher during spring, winter and autumn seasons, than summer season. Numbers of the normal follicles were significantly ($P < 0.05$) higher during spring, while the atretic follicls were significantly ($P < 0.05$) higher during summer season than other seasons. Oocytes recovery, compact oocytes complexes (COC's) and partially denuded cumuls oocytes (PDCO) were significantly ($P < 0.05$) higher during autumn, while expanded cumulus oocytes (ECO) and denuded cumulus oocytes (DCO) were significantly ($P < 0.05$) higher during spring and winter seasons than other seasons of the year. The highest ($P < 0.05$) activities of follicular fluid aspartate – aminotransaminase (AST), alanine – aminotransaminase (ALT), alkaline phosphatase (ALP) and acid phosphatase (ACP) enzymes were recorded during summer and the lowest ($P < 0.05$) activity was recorded during spring season. The highest ($P < 0.05$) values of follicular fluid potassium and calcium were recorded during winter and the lowest ($P < 0.05$) values were recorded during summer season. Testosterone concentration was significantly ($P<0.05$) higher, however cholesterol concentration was significantly ($P < 0.05$) lower during summer season, meanwhile oestradiol-17β concentration was significantly ($P < 0.05$) higher during winter season than other seasons of the year. Ovary weight, number of the corpora lutea (CL) and number of the normal follicles in the left were significantly ($P< 0.05$) higher than the right ovary, while the number of the atretic follicles in the right was significantly ($P<0.05$) higher than the left ovary. Oocyte recovery and oocyte status (COC's, PDCO, ECO and DCO) in the left ovary were significantly ($P < 0.05$) higher than the right one. In respect to ovary side, AST, ALT, ALP, ACP, sodium and testosterone concentration of follicular fluid in the left ovary were significantly ($P < 0.05$) lower than the right one. Cholesterol, potassium, calcium, inorganic phosphorus and oestradiol-17β concentrations in the left were significantly ($P < 0.05$) higher than the right ovary. In the second experiment, results revealed significantly ($P<0.05$) higher cumulus expansion, meiosis	Full Text	61

	metaphase I (MI) and metaphase II (MII) than the non-breeding season . When the type of culture media there was no differences in cumulus expansion except with basal medium which produce the lowest incidence in both breeding and non-breeding season. In breeding season, TCM-199 medium showed the highest rate ($P<0.05$) of MII oocytes, while in non-breeding season, TCM-199 and Ham's F-10 media showed the highest rates ($P<0.05$) of MII oocytes. [A.E.B. Zeidan, M.A. El-Harairy, Sh.A. Gabr, M.A. Tag El-Dien, S. A. Abd El-Rahman and A.M. Amer. ***In Vitro* Maturation of Camel Oocytes As Affected By Different Media during Breeding and Non-Breeding Seasons**. Journal of American Science 2011;7(1):460-472]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words:** Camels, season, ovary, follicular fluids, oocytes, *in vitro* maturation		
62	**Synthesis And Evaluation Of Novel Cationic Monomers Viscosifiers For Oil Well Drilling Fluids** A.M., Badwi, M. M., Dardir* and H. M., Ahmed Egyptian petroleum research institute EPRI, NASR CITY 11727, CAIRO EGYPT monamdardir@yahoo.com **Abstract:** Novel cationic monomers capable of forming viscoelastic fluid were prepared. The monomers were formed through the quternzation reaction of allyl halides with dimethylalkylamines, triethanolamine or N-N dimethyl aniline. The chemical structures of the prepared monomers were conformed using FTIR and H^1NMR spectroscopy. The result of the spectroscopic analysis indicate that they were prepared through right method they have high purity and there surface properties were studied. The cationic monomer products were evaluated as viscosifiers and filter loss additives for water –base mud because they were capable of forming viscoelastic fluids in high brine solution. Rheological properties, gel strength, filter loss and thermal stability of the water- based mud formulated with the new cationic monomers were studied compared to the commercial viscosifier (reference sample mud). [A.M., Badwi, M. M., Dardir and H. M., SYNTHESIS and EVALUATION of NOVEL CATIONIC MONOMERS VISCOSIFIERS for OIL WELL DRILLING FLUIDS. Journal of American Science 2011;7(1):473-484]. (ISSN: 1545-1003). http://www.americanscience.org. **Keyword:** Drilling fluids-Viscosifier-Rheological properties	Full Text	62
63	**Effect of ripening conditions on the properties of Blue cheese produced from cow's and goat's milk** EL-Sheikh, M.M.; M.H. EL-Senaity; Y.B. Youssef and Nadia M. Shahein and N.S. Abd Rabou Dairy Department, National Research Centre, Dokki, Cairo, Egypt mmorsy57@yahoo.com **Abstract:** Blue cheese (style Roquefort) was made from cow's and goat's milk. Fresh cheese was ripened at room conditions for 30 days, then resulted cheese were divided into two portions, one was complete ripened at room conditions and the other was complete ripened at refrigerator for another 30 days. Cheese samples were analyzed at 1, 30 and 60 days of ripening period, for moisture, fat, pH, total nitrogen and free amino acids. Tyrosine & Tryptophan and total volatile fatty acids contents as well as their organoleptic properties. No clear differences were observed between both goat's and cow's cheese in their gross composition. Goat's blue cheese ripened for 60 days at room temperature had a higher total free amino acids contents than that in cow's cheese, while their values were higher when cheese ripened at refrigerator than that ripened at room temperature. Blue cheese from goat's milk showed the highest total volatile fatty acids and Tyrosine & Tryptophan contents during ripening, at the end of ripening, the cheese ripened at room temperature gave the higher values than that ripened at refrigerator. Blue cheese from goat's milk ranked a higher score for organoleptic properties during ripening conditions compare with that made from cow's milk. It can be concluded that goat's milk can be successfully used in the manufacture of blue cheese and ripened at room temperature with high quality over than that from cow's milk. [EL-Sheikh, M.M.; M.H. EL-Senaity; Y.B. Youssef and Nadia M. Shahein and N.S. Abd Rabou. **Effect of ripening conditions on the properties of Blue cheese produced from cow's and goat's milk**. Journal of American Science 2011;7(1):485-490]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Blue cheese, Cow's Goat's milk	Full Text	63

64	**Manufacture of Cultured Butter Milk Beverage from Whole and Skimmed Goat's Milk** Youssef, B.Y.; M.H. El-Senaity; M.M. El-Sheikh; N.S. Abd-Rabou and Nadia, M. Shahein Dairy department, National Research Centre, Dokki, Cairo, Egypt. ns_abdrabou@yahoo.com **Abstract:** The development of high quality cultured butter milk beverage (CBMB) is primarily dependent on a controlled fermentation of the milk constituents. Cultured butter milk beverage was made from either whole or skim goat's milk, using mesophilic L-starters FR 19-8126 (Lacfococcus lactis subsp.lactis, Lact. cermohs subsp.cremoris and Leuconostoc cremohs) and DL-starters A-8101 (the same of microorganisms L-starters contain plus Lact. lactis Subs, diacetilactis). Chemical, flavour and organoleptic properties of the resultant four CBMB treatments were compared, when fresh and during 15 days of storage at 7°C. The CBMB made from goat's whole milk cultured with DL-starters had diacetyl and acetaldehyde values which were reported to be necessary for a good flavour balance. Moreover, it received the highest organoleptic scores. Therefore, this CBMB was recommended to be produced commercially in Egypt. [Youssef, B.Y.; M.H. El-Senaity; M.M. El-Sheikh; N.S. Abd-Rabou and Nadia, M. Shahein. **Manufacture of Cultured Butter Milk Beverage from Whole and Skimmed Goat's Milk**. Journal of American Science 2011;7(1):491-497]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Cultured butter milk, Goat's milk, L- starter, DL-starter	Full Text	64
65	**Well Logs Application in Determining the Impact of Mineral Types and Proportions on the Reservoir Performance of Bahariya Formation of Bassel-1x Well, Western Desert, Egypt.** Tarek F. Shazly and Mohamed A. M. Ramadan* Egyptian Petroleum Research Institute, Cairo, Egypt. moh_ramadan2222@yahoo.com * **Abstract:** The present work dealt with the computerized well log analysis of Bassel – 1X well in the Sherouk Field in the Northern Western Desert of Egypt to determine the mineralogical composition of Lower and Upper Bahariya Formation and to estimate the influence of these minerals on the different petrophysical parameters of Lower and Upper Bahariya Formation. The lithologic and mineralogical compositions were identified qualitatively through the utilizing of crossplots which were established by using the different petrophysical parameters. Also the lithologic and mineralogical compositions were established quantitatively by using the mathematic equations. The matrix components of Lower Bahariya Formation included few percentage of clay minerals (illite and montmorillonite) and high quantity of quartz, calcite and dolomite, while in Upper Bahariya Formation it involves high percentage of clay minerals (illite and montmorillonite) and low quantity of quartz, calcite and dolomite. These minerals were plotted against the different petrophysical parameters to show the effect of these minerals on the effective porosity and the saturation of hydrocarbon. [Tarek F. Shazly and Mohamed A. M. Ramadan. **Well Logs Application in Determining the Impact of Mineral Types and Proportions on the Reservoir Performance of Bahariya Formation of Bassel-1x Well, Western Desert, Egypt**. Journal of American Science 2011;7(1):498-505]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Logs; Mineral Type; Reservoir; Bahariya; Western Desert; Egypt	Full Text	65
66	**Comparison between Molecular and Classical Techniques for Identification of *Mycoplasma* species Isolated from Mastitic Ruminants.** [1]Hassan, W.H.; [2]Mona, A. El-Shabrawy; [2*]Hakim, A.S.; [2]Azza, S.M. Abuelnaga; [2] Samy A. A and [2]Sadek E. G. [1] Bact. Mycol. and Immuno. Dept. Vet. Med. Beni-Suef University, Beni-Suef, Egypt [2]Microbiol. and Immuno. Dept. National Research Centre, Cairo, Egypt migris410@yahoo.com* **Abstract:** 165 cows and 19 buffaloes were examined to detect the *Mycoplasma* mastitis, the result revealed	Full Text	66

	that 114 (69.59%) and 6 (31,57%) were clinically mastitic cows and buffaloes respectively while 51 (30.9%) and 13(68.42%) were apparently healthy cows and buffaloes respectively .On examining the apparently healthy cows and buffaloes, the result were 67 (32.84%) and 18 (34.61%) from subclinically mastitic cows and buffaloes respectively while 137(67.15%) and 34 (65.38%) fro apparently completely healthy. *Mycoplasma* were isolated in percentages of 8.9%, 5.5% from subclinically mastitic cow and buffaloes respectively and in percentages of 12.97%, 12.5% from clinically mastitic cows and buffaloes respectively. *M. bovis* was isolated from 8 (32%) and M. bovigenitalium was in percentage of 7 (28%) and the unidentified *Mycoplasma* was 10 (40%). Isolation of *Mycoplasma* from udder tissue in cows and buffaloes were in percentage of 2 (28.5%) in cows while no *Mycoplasma* isolates were obtained from buffaloes udder tissues. Application of PCR technique on these isolates and some negative samples, these were positive with percentage 100%. On the other hand, 192 sheep and 118 goats were examined. We found that in percentage of 82 (42.7%) and 43 (36.44) from sheep and goats respectively were clinically mastitic. Isolation of *Mycoplasma* was in percentage of 11 (13.41%) and 17 (39.53%) of sheep and goat respectively. Identification of these isolates revealed 8 (29%) was *M. agalactia* isolates and 20 (71%) was unidentified *Mycoplasma* spp. Application of PCR technique on *M. agalactia* isolates which identified by traditional techniques by use specific primers to *M. agalactia* revealed negative results but on using the primer specific to *M. bovis* to the same isolates, it was positive to all isolates 8 (100%). [Hassan, W.H.; Mona, A. El-Shabrawy; Hakim, A.S.; Azza, S.M. Abuelnaga; Samy A. A and Sadek E. G. **Comparison between Molecular and Classical Techniques for Identification of *Mycoplasma* species Isolated from Mastitic Ruminants**. Journal of American Science 2011;7(1):506-613]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** *Mycoplasma bovis*; *Mycoplasma agalactia*; mastitis; PCR		
67	**Molecular and Virulence Characterization of *Escherichia.coli* strains Isolated from Persistent Bovine Mastitis.** [1]Salwa, M. Helmy; [2]Ammar, M. A.; [3]Aisha R. Ali; [4]Mona, A. El-Shabrawy; [4*]Hakim.A.S.; [4] Bakry, M.A.; [4]Azza, S.M. Abuelnaga and[4]Eraqi, M. M. [1]Bacteriology, Mycology and Immunology Department Faculty of Veterinary Medicine Kafrelsheikh University, [2] Microbiology, Faculty of Veterinary Medicine, Zagazig University, Zagazig , Egypt [3] Serology Unit Animal Health Research Institute Dokki, Giza, Egypt [4]Microbiology and Immunology National Research Center, Dokki, Giza, Egypt migris410@yahoo.com [*] **Abstract:** Four hundred and fifty lactating cows were examined according to the clinical observation and the California mastitis test, 181 were clinical mastitis with the percentages of 40.2%, and revealed 57 *E.coli* isolates, the incidence of clinical mastitis is higher in hind quarters (63.97%) than the fore quarters (36.02%). Serotyping of *E.coli* revealed 8 different serovars of *E.coli* according to somatic antigen O55 (19.2%), O111 (15.8%), O124 (12.3%), O119 (12.3%), O114 (10.5%), O26 (7%), O157 (7%) and O44 (3.5%), in addition, (12.2%) of isolated *E.coli* strains could not be serologically identified by the available antisera. The incidence of recurrent *E.coli* mastitis, 26.3% (15 of 57) occurred in 5 of 56 quarters 8.9% of 5 cows, the most *E.coli* serogroups recovered from recurrent *E.coli* mastitis from 5 quarters of 5 cows were O55, O119, O111, and O157. The adherent and invasive property were the most common factors in *E.coli* serogroups (O55, O119, O111 and O157) which were isolated from recurrent mastitis and give positive results with (*eae*A) gene but it is less in *E.coli* serogroups(O124, O114, O26 and O44) which give negative results with (*eae*A) gene**.** [Salwa, M. Helmy; Ammar, M. A.; Aisha R. Ali; Mona, A. El-Shabrawy; Hakim.A.S.; Bakry, M.A.; Azza, S.M. Abuelnaga and Eraqi, M. M. **Molecular and Virulence Characterization of *Escherichia.coli* strains Isolated from Persistent Bovine Mastitis**. Journal of American Science 2011;7(1):614-624]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words:** Recurrent mastitis- adherent *Escherichia coli*-intimin	Full Text	67
68	**Hydrochemistry and levels of some heavy metals in samples of Ibeshe, Lagos Lagoon Complex, Nigeria** Ladigbolu Ismail Adejare, Balogun Kayode James and Shelle R.O.	Full Text	68

	Nigerian Institute for Oceanography and Marine Research, 3 Wilmot point, Bar beach Victoria Island, Lagos, Nigeria Corresponding Author: ladadejare@yahoo.com **ABSTRACT:** The concentration of Iron, Copper, Chromium, Nickel, Lead, Manganese, Arsenic, Cadmium and Zinc were determined in the surface water, sediments and fish samples (*Chrysichthys nigrodigitatus*) of an industrial effluent receiving water in Ibeshe, Lagos Lagoon Complex between February and June, 2009. In assessing the impact of effluent discharge on the lagoon, Water and fish samples result were compared with the WHO/FEPA standard while the sediments results were compared with the results for unpolluted sediment. The average levels of heavy metals found in surface water, sediment and fish samples were as follows: surface water; 0.293mg/l for Fe, 0.177mg/l for Cu, 0.107mg/l for Pb, 0.213mg/l for Cr, 0.177mg/l for Mn, 0.233mg/l for Ni and <0.10mg/l for Cd. Sediment; 85303.33µg/g for Fe, 53.967µg/g for Cu, 38.35µg/g for Pb, 110.183µg/g for Zn, 93.88µg/g for Cr, 274.967 µg/g for Mn, 1.017 µg/g for As, 67.4 µg/g for Ni and 1.00µg/g for Cd. Fish sample; 4.263 µg/g for Fe, 8.229µg/g for Cu, 1.967µg/g for Pb, 11.338µg/g for Zn, 1.329µg/g for Cr, 1.513µg/g for Mn, 4.046µg/g for Ni and 0.458µg/g for Cd. The concentration of Pb and Ni in surface water were higher than WHO / FEPA limits, while Cd, Zn, Cr and As were found below FEPA limit. Fe, Cu, Pb, Cd and Zn were all higher in concentration when compared with the values of unpolluted sediment. Consequently, the concentration of Zn, Cr, Cd in fish were below the FEPA limit. Water quality of Ibeshe were typify of alkaline pH (8.90 - 9.00), high Dissolved Oxygen content (4.20 -7.80mg/l), Turbidity (24.8 – 156NTU) and freshwater salinity values (0‰). The findings reported in this study would be expected to serve as baseline level for future heavy metal pollution status of the Ibeshe, Lagos Lagoon area. [Ladigbolu Ismail Adejare , Balogun Kayode James and Shelle R.O. **Hydrochemistry and levels of some heavy metals in samples of Ibeshe, Lagos Lagoon Complex, Nigeria**. Journal of American Science 2011;7(1):625-632]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords**: Lagoon, effluent discharge, sediment, Heavy metal		
69	**Breeding success of Lesser Crested Tern and Swift Tern at Shidvar island, Iran** Saber Ghasemi[1], Farhad Hosseini Tayefeh[2], Neda Mola Hoveizeh[3] [1]Faculty of Environmental science, Islamic Azad University, Bandar abbas Branch, Iran. Tel:(+98) (761) 6672328, Mobile:(+98)935-820-1684, E_mail:saberghasemi@gmail.com [2]Department of Environment, Bushehr Province, Iran. Tel:(+98) (917)7755886, E_mail:farhadtayefeh@gmail.com [3]Faculty of Environmental science, Islamic Azad University, Bandar abbas Branch, Iran. Mobile :(+98) 937-355 7610, E_mail:neda7975@yahoo.com **ABSTRACT:** The aim of this study was to investigate the breeding success of Lesser Crested Tern *Sterna bengalensis* and Greater Crested Tern *Sterna bergii* at Shidvar Island, in Persian Gulf, southern of Iran. Total Count Method that included tree breeding colonies was carried out. A total of 365 nests, belonging to 240 nest of Lesser Crested Tern and 125 nest of Swift Tern, were categorized under number of eggs and were counted. The mean clutch sizes of Lesser Crested Tern and Swift Tern were estimated 1.04±0.01 and 1.04±0.03 respectively. Furthermore, the average of breeding success during incubation of eggs, nestling and post-nestling were measured 67.7%, 100% and 95.24% for Lesser Crested Tern and 83.3%, 70% and 100% for Swift Tern. The total breeding success was measured 74.43% and 66.63% for them respectively. Relative abundance of birds during outward migration was measured 65.32% and 34.68% for two species, respectively. It is considered that the importance of Shidvar Island for seabirds, especially for family of Sternidae, must be recognized and the protection of this site from threats must be enforced. [Saber Ghasemi, Farhad Hosseini Tayefeh, Neda Mola Hoveizeh. **Breeding success of Lesser Crested Tern and Swift Tern at Shidvar island, Iran**. Journal of American Science 2011;7(1):633-638]. (ISSN: 1545-1003). http://www.americanscience.org. **KEYWORDS:** Breeding Biology, *Sterna bengalensis*, *Sterna bergii*, Shidvar, Iran	Full Text	69
70	**Transmissivity of the Glazing Surface of a Solar Flat Plate Collector Based on the Metrological Parameters of Yola, Nigeria** Bello Y Idi[1] and Dillip K De[2]	Full Text	70

	[1]Department of Physics, Adamawa State University, Mubi Nigeria Belyus2000@gmail.com [2]Department of Physics, Federal University of Technology, Yola, Nigeria Dipak61@yahoo.com **Abstract:** A glazing surface is one of the most vital components of a solar flat plate collector which is meant to admit maximum possible radiation and minimizes upward loss of heat. The most commonly used glazing surface is transparent glass. The performance of the glazing surface depends on the magnitude of its transmissivity. For a given material, this optical property is a function of solar geometry that varies with geographic location. In this work, the monthly mean value of transmissivity of the most commonly used glazing surface, 3mm transparent glass was determined for 12 months of the year with respect to the solar geometry of Yola town. A peak value of 0.8823 was recorded in the month of September while a minimum value of 0.8775 was recorded in the month of January. An annual mean value of 0.8807 was recorded with a standard deviation of 0.0015. The results imply that plane glass as a glazing surface admits about 88% of the solar radiation incident on it to the absorbing surface. The slight variation all year round is an indication of its consistent performance all times of the year at the locality. [Bello Y Idi and Dillip K De. **Transmissivity of the Glazing Surface of a Solar Flat Plate Collector Based on the Metrological Parameters of Yola, Nigeria**. Journal of American Science 2011;7(1):639-643]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words:** transmissivity, plane glass, glazing cover, flat plate collector, solar energy		
71	**Utility Mapping with Ground Penetrating Radar: an Innovative Approach** Bello. Y. Idi[a] and Md. N. Kamarudin[b] [a]Department of Geomatic Engineering, FKSG, Universiti Teknologi Malaysia. belyus2000@gmail.com [b]Institute of Geospatial Science and Technology, (INSTEG), Universiti Teknologi Malaysia. mdnorkamarudin@utm.my **Abstract:** A new approach for the fitting of hyperbolic signatures due to point or cylindrical reflector in a GPR radargram is proposed. The technique is based on the least square error minimization of hyperbolic function derived from the general equation of hyperbola leading to the determination of the optimal values of the fitting parameters at the minimal level of sum of squared error function. The parameters are used to determine the radar velocity and the radius of cylindrical reflector. A test for the effectiveness of the proposed technique was conducted using a GPR radargram obtained at a road side where subsurface utilities are anticipated. A unique hyperbolic signature obtained in the radar image was digitized and interpreted using the developed algorism in MATLAB environment. Hyperbolic fitting parameters *a* and *b* were numerically obtained as 49.6444ns and 4.3182m respectively. The parameters were used to obtain the media velocity, dielectric constant and depth of the reflector as 0.174m/ns, 2.973 and 2.61m respectively. The technique therefore seems promising and a new approach to utility mapping. [Bello. Y. Idi and Md. N. Kamarudin. **Utility Mapping with Ground Penetrating Radar: an Innovative Approach**. Journal of American Science 2011;7(1):644-649]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords**: Ground penetrating radar, least square fitting, radar velocity, hyperbolic reflection, utility mapping	Full Text	71
72	**Application of variational iteration method for solving the nonlinear generalized Ito system** A.M. Kawala *; Hassan A. Zedan ** *Department of Mathematics, Faculty of Science, Helwan University, Cairo, Egypt **Department of Mathematics, Faculty of Science, Kafer el sheik University, Cairo, Egypt **kawala_26_1@yahoo.com** **Abstract:** In this article, we implement relatively analytical technique called the variational iteration method (VIM)for solving nonlinear generalized Ito system. In this method, a correction functional is	Full Text	72

	constructed by a general Lagrange multiplier. Two cases are given to illustrate the accuracy and effectiveness of the method .We compare our results with results obtained by exact solution. This Comparison reveals that the variational iteration method is very effective, convenient and easier to be implemented. [A.M. Kawala; Hassan A. Zedan. **Application of variational iteration method for solving the nonlinear generalized Ito system**. Journal of American Science 2011;7(1):650-659]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Variational iteration method; Lagrange multiplier; nonlinear generalized Ito system		
73	**Abundance Of Molluscs (Gastropods) At Mangrove Forests Of Iran** S. Ghasemi[1], M. Zakaria[2], N. Mola Hoveizeh [3] [1]Faculty of Environmental science, Islamic Azad University, Bandar abbas Branch, Bandar abbas, Iran. Tel: (+98) 9397231177, E_mail:saberghasemi@gmail.com [2]Faculty of Forestry, University Putra Malaysia, Malaysia. [3]Faculty of Environmental science, Islamic Azad University, Bandar abbas Branch, Iran. **ABSTRACT:** This study determined the abundance and diversity of molluscs (focused on gastropod) at Hara Protected Area (HPA) and Gaz and Hara Rivers Delta (GHRD) mangroves, southern of Iran. Point count sampling method was employed in this study. A total of 1581 individual of gastropods, representing 28 species and 21 families, were observed in the two sites. The PCA plot indicated that all species have correlation with winter excluding species namely *Ethalia sp.*, *Haminoea sp.*, *Trichotropis sp.* and *Tibia insulaechorab curta* at HPA and *Telescopium telescopium*, *Stocsicia annulata*, and *Stenothyra arabica* at GHRD. The mean number of species was estimated 6.88±2.77 (per plot) versus 9.65±6.63 (per plot) at HPA and GHRD respectively. The results of X^2 test indicated that there was a high significant difference between total gastropod population observed at 4 seasons ($X^2_{3,1}$=31.9, p<0.001), but there was no significant difference in term of number of species between sites in order to seasonal observation ($X^2_{3,1}$=0.84, p>0.05). The results of diversity comparisons indicated that the highest diversity was in the HPA as compared to GHRD. Furthermore, the SIMPER analysis indicated that mangroves of HPA and GHRD were dominated with Asseminea sp., although the number of population was much higher at *R. mucronata* habitat. Eight species namely *Asseminea sp.*, *Stenothyra arabica*, *Cerithidium cerithinum*, *Littoria intermedia*, *Telescopium telescopium*, *Iravadia quadrasi*, *Atys cylindrica* and *Cyclostrema ocrinium* represented more than 91% of observations at HPA, while at GHRD, there were only three species namely *Asseminea sp.*, *Stenothyra arabica* and *Cerithidea cingulata* which represented more than 90% of observations. The result states that the great importance of HPA and GHRD for gastropod assemblages as main food resource for wading birds must be recognized and the protection of these sites from threats must be thoroughly enhanced. [S. Ghasemi, M. Zakaria, N. Mola Hoveizeh. ABUNDANCE OF MOLLUSCS (GASTROPODS) AT MANGROVE FORESTS OF IRAN. Journal of American Science 2011;7(1):660-669]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words:** Gastropod, Mangrove Forest, Abundance, Iran	Full Text	73
74	**Barriers of Local Participation in Rural Cooperatives A Case Study of Fars, Iran** Abrisham Aref School of Humanities and Social Science, Science and Research Branch Islamic Azad University, Tehran, Iran abrishamaref@yahoo.com **Abstract:** Local participation has an important role in development of rural cooperatives. This article attempts to illustrate the barriers of people participation in rural cooperatives in Fars Province, Iran. Rural cooperatives are certainly a major contributor to rural development in many countries. But, in this case there are a significant number of barriers to effectively using rural cooperatives as a tool for rural development. This paper used qualitative approach to illustrated barriers of cooperatives through local participation. The findings through focus group identified several constraints that have limited active local participation in rural cooperatives.	Full Text	74

	[Abrisham Aref, **Barriers of Local Participation in Rural Cooperatives A Case Study of Fars, Iran**. Journal of American Science 2011; 7(1):670-673]. (ISSN: 1545-1003). http://www.americanscience.org **Keywords:** participation, rural cooperatives, rural development		
75	**Biofilm Formation by Blood Stream Staphylococcal Isolates from Febrile Pediatric Cancer Patients at South Egypt Cancer Institute** Salwa S. Seif El-Din[*1], Moustafa S. El-Rehewy[1], Mohammed M. Ghazaly[2], Mohamed H. Abd-Elhamid[3] Medical Microbiology and Immunology[1], Pediatric Oncology[2] Departments, Faculty of Medicine Assuit University and Clinical Pharmacy at South Egypt Cancer Institute[3], Assiut, Egypt *salwaegy@yahoo.com **Abstract:** Background Blood Stream infection (BSI) remains the major cause of morbidity and death in patients undergoing treatment for cancer. Approximately 10% to 30% of all febrile neutropenic cancer patients are bacteremic at presentation. Staphylococci are the most frequently isolated organisms from blood cultures of febrile neutropenic (FN) cancer patients. Aims: This study aimed to define the main causative organisms of 139 episodes of bacteremia in 100 febrile neutropenic pediatric patients admitted to South Egypt Cancer Institute (SECI), pediatric oncology ward. Also to study the prevalence of biofilm forming capability of the coagulase-negative staphylococci (CONS) and *Staphylococcus aureus* (*S. aureus*) blood isolates (39) (group A) and their relation to clinical and in 29 staphylococci strains and nasal mucosal isolates from healthy care workers (group B). Methods: All Isolates were identified and tested for antibiotic susceptibility by Micrscan Walkaway System. The CONS and *S. aureus* isolates from blood cultures of pediatric patients were then tested for slime production using qualitative congo red agar plate test (CRA test), quantitative microtitre plate assay (MTP). The presence of *icaA* and *icaD* genes by polymerase chain reaction (PCR) was also determined. Results: Among 139 episodes of fever and neutropenia recorded in 100 patients, bacteremia represented 54.7% in which Gram negative organisms constituted 53 % from the total episodes obtained and Gram positive staphylococcal isolates were 47%. *S. aureus* were 14 strains and CONS were 22 strains. Of the 14 *S. aureus*, 10 strains were *icaA* and *icaD* positive versus 8 strains were CRA test positive and also were MTP positive. Two strains of *S. aureus* were PCR positive for *ica* genes and slime negative on CRA and MTP. Of the 22 CONS, 12 (53%) were *ica* genes positive versus 11 strains (46%) were positive using CRA test and 9 strains were MTP positive. One strain of CONS was positive using MTP and PCR negative. Group B isolates were CRA, MTP and *ica* genes negative. Biofilm forming staphylococcal strains on CRA (15/19) and (16/22) with *ica* genes were resistant to Imipenem, Amoxicillin/clavulanic, Cephlosporins, and Oxacillin. Conclusions: The results of the present study shows a high percent of Gram negative bacteremia in pediatric oncology ward and that isolates expressing *ica* genes were exhibiting more resistance to broad spectrum antibiotics. This supports that biofilm adds to the virulence profile isolated from blood stream infections and that the *ica* genes are important virulence markers for clinically significant CONS isolates. The better agreement between the CRA plate tests with the molecular detection of *ica* genes indicates the former as a reliable test for the phenotypic characterization of virulence of clinical isolates. [Salwa S. Seif El-Din, Moustafa S. El-Rehewy, Mohammed M. Ghazaly, Mohamed H. Abd-Elhamid. **Biofilm Formation by Blood Stream Staphylococcal Isolates from Febrile Pediatric Cancer Patients at South Egypt Cancer Institute**. Journal of American Science 2011;7(1):674-686]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Biofilm, bacteraemia, coagulase negative staphylocci, cancer	Full Text	75
76	**Prevalence of SEN Virus Infection in Multitransfused Patients in Assiut University Hospitals, Egypt** Ismail S. Mohamed [1], Amany G. Thabit [1], Sherine A. Abd-El Rahman[1], Essam Eldin A.Mohammed [2], Salwa S. seif Eldin[*1] and Aliaa M. A. Ghandour [1] Departments of Medical Microbiology& Immunology [1] and Internal Medicine [2], Faculty of Medicine, Assiut University, Assiut , Egypt *salwaegy@yahoo.com **Abstract:** Background*:* SENV is a blood- borne, circular ss DNA virus and possessing nine genotypes (A	Full Text	76

	to I).Among nine genotypes, SENV-D and SENV-H genotypes have the strong link with patients with non (A-E) hepatitis infections. Recently, the identification of SEN virus (SENV) as a possible etiologic agent of parenteral transmission hepatitis let to the study of the prevalence of such agent. This study compared SENV prevalence and its two important genotypes (D&H) which might be pathogenic in high risk subjects including blood transfused patients and hemodialysed patients and low risk subjects as healthy blood donors. Subjects and methods: This study included 75 multitransfused patients, 60 of them were hemodialysed and the remaining were blood transfused including haemophilics, anaemics and leukemics. The study included also 25 healthy blood donors as a control. They were enrolled consecutively at the department of Internal Medicine, Assiut University Hospital. The sera were separated and SENV DNA was detected by polymerase chain reaction. Results: A higher prevalence of SENV infection was detected *in* patients groups than in blood donors (46.7% versus 20%).No significant relation was found between SENV infection and age, duration of haemodialysis or liver enzymes. However, there was significant difference between SENV positive and negative patients as regards gender and number of blood transfusions. Conclusion: SENV is commonly present in blood transfused and haemodialysed patients attended to Assiut University Hospitals as well as in blood donors at comparable rates. SENV infection has been found in only 20% of blood donors but in 46.7% of patients. The results also indicated that other possible routes of SENV infection other than blood transfusion may be included. Its pathogenic role in causing hepatitis is not documented, so far it can be considered as simple guest till further studies have been done. [Ismail S. Mohamed, Amany G. Thabit, Sherine A. Abd-El Rahman, Essam Eldin A.Mohammed, Salwa S. seif Eldin and Aliaa M. A. Ghandour. **Prevalence of SEN Virus Infection in Multitransfused Patients in Assiut University Hospitals, Egypt**. Journal of American Science 2011;7(1):687-696]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Prevalence; SEN; Virus; Infection; Patient		
77	**The Effectiveness of Kangaroo Technique on Preterm Baby Weight Gain** Iman Ibrahim Abd El Moniem and Madiha Amin Morsy* Child Department, Faculty of Nursing - Ain Shams University, Cairo, Egypt *madihaaboughalaa@yahoo.com **Abstract:** The aim of the study was to assess mother's perception about kangaroo technique, implement on hospitalized premature babies and evaluate the effectiveness of kangaroo technique on preterm babies weight gain. A quasi experimental design was used in this study. The study subjects consisted of two hundred (200) mothers divided into two identical groups. The studied group included mothers who applied the kangaroo technique, while those exposed to routine hospital care were consider a control. Data were collected through using pre-designed interviewing questionnaire to assess mothers and neonates characteristics, knowledge about kangaroo technique. An observational checklist was used to assess mothers' practices; towards application of kangaroo technique. This technique had been applied for the study group only. The result of the study revealed that there was a statistically significant difference in mother's knowledge and practices between both study and control groups after application of kangaroo technique with significant effects on preterm baby weight and attachment. The study concluded that application of kangaroo technique enhanced mother-child attachment and had positive effect on weight gain and possibility of early discharge from neonatal intensive care units (NICUs). Therefore, the study recommended the application of kangaroo technique for all low birth weight premature babies as part of the routine daily care to babies admitted to the neonatal intensive care units. [Iman Ibrahim Abd El Moniem and Madiha Amin Morsy. **The Effectiveness of Kangaroo Technique on Preterm Baby Weight Gain**. Journal of American Science 2011;7(1):697-702]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Kangaroo technique- Premature babies-Mother infant bonding-Duration of hospitalization-weight gain	Full Text	77
78	**The Contribution of Women in Rural Development in Iran** Fatemeh Allahdadi School of Humanities and Social, Science and Research Branch Islamic Azad University, Tehran, Iran, faaref@yahoo.com	Full Text	78

	Abstract: This paper highlights the concerns of women and the challenges they face in rural development process. Agriculture is certainly a major contributor to rural development in many countries. It is one of the most important economic sectors in Iran. In this way rural women play a special role in rural development. When women are economically and socially empowered, they can become a potent force for change. Findings through secondary data showed that although women have an important role in rural development in Iran, but there are some problem faced by women farmers. The finding can assist the local organizations and community developers for remove this problem**.** **[**Fatemeh Allahdadi. **Enhancing the Role of Women Farmers in Rural Development.** Journal of American Science 2011;7(1):703-707]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words:** women farmers, rural development, agricultural development		
79	**Simultaneous diffusion of Cr-Si on Ni-Base super alloy using pure Cr and Si by pack cementation method** A. Afshar[a], A. Sabour[b], M. Saremi[c] , D. Ghasemi[a,*] [a] Islamic Azad University, Science and Research Branch, Tehran, Iran, [b] Tarbiyat Modarres University - Tehran – Iran, [c] Department of Materials Science and Engineering -Tehran University – Tehran – Iran. *** Corresponding Author:** Davood Ghasemi, E-mail: Davoodghasemi@yahoo.com **Abstract:** Pure Cr and Si powders were used to produce Cr-Si coatings by Simultaneously diffusion of these elements on Ni-base Super alloy. A mixture of elemental Cr and Si powders (as Cr, Si sources) was used with (NaCl-NaF) or (NaCl-NaF-NH_4Cl) mixed activators were applied. The results of this study indicated that for co diffusion of these elements, Si content must be 0.1 Cr content in the pack mixture. Using 95%NaCl-5%NaF mixed activator was produced porous Cr-Si coatings, but by addition of 1% NH_4Cl to pack mixture, porosity of Cr-Si coating was eliminated. Increasing of (NaCl-NaF) content was leaded to increase depth of Si diffusion into the surface. [A. Afshar, A. Sabour, M. Saremi, D. Ghasemi. **Simultaneous diffusion of Cr-Si on Ni-Base super alloy using pure Cr and Si by pack cementation method.** Journal of American Science 2011;7(1):708-711]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words:** Simultaneous diffusion, pack cementation, pure elements, mixed activator, Super alloy.	Full Text	79
80	**Effect of some Strains of Probiotic Bacteria against Toxicity Induced by Aflatoxins *in vivo*** Abou-Baker Salim[1], Azza Zohair[2], Amany El-Saied Hegazy[3] and Amal Said[3] [1]Food Toxicology and contaminants Department, National Research Center, [2]Faculty of Specific Education, Minufiya University, [3]Nutrition Department, National Research Center, Cairo, Egypt [*]salimali740@hotmail.com **Abstract:** Aflatoxins are highly toxic, mutagenic, teratogenic and carcinogenic compounds produced by some species of Aspergillus, especially *A. flavus* and *A. parasiticus.* This study was conducted investigate the effect of some strains of probiotic bacteria against toxicity induced by contaminated diet with aflatoxins in male rats. Animals were divided into 6 equal groups each group contains 7 rats. The first group received a basal diet and served as negative control, the second group received basal diet supplemented with strain 1 of probiotic bacteria (*Bifidobacterium bifidum),* the third group received basal diet supplemented with strain 2 of probiotic bacteria *(Lactobacillus acidophilus),* the fourth group received basal diet supplemented with 1.34ppm aflatoxins contaminated peanut as positive control group. The other two groups received basal diet supplemented with 1.34ppm aflatoxins contaminated peanut plus strain 1and strain 2 probiotic bacteria for 6 weeks. Results revealed that positive control gave a very significant increased in alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline Phosphatase (ALP) activities, creatinine and urea; while decreased total protein (TP), albumin and globulin indicating the toxicity of aflatoxin on both liver and kidney functions. However probiotic strains supplemented to aflatoxins treated group revealed a significantly alleviated TP, albumin and globulin depletion in serum with an elevation of ALT, AST, ALP, creatinine and urea levels. Results also showed that the group received basal diet supplemented with strain 1 (*Bifidobacterium bifidum)* and with strain 2 *(Lactobacillus acidophilus)* showed significant beneficial health effects. It was noticed that the group received *Lactobacillus acidophilus* showed better results than *Bifidobacterium bifidum.* Results indicated also that	Full Text	80

	the protective action of probiotic strains as a potential protective agent against aflatoxin toxicity as well as their beneficial health effects and may thereby offered an effective dietary approach to decrease the risk of occurrence of liver, kidney function and occurrence of cancer which may be due the ability of probiotic strains to bind with aflatoxins, reduced their uptake, and protected against both memberane and DNA damage. The study revealed also that probiotics can also provide benefits by modulating immune functions. **[**Abou-Baker Salim, Azza Zohair, Amany El-Saied Hegazy and Amal Said. **Effect of some Strains of Probiotic Bacteria against Toxicity Induced by Aflatoxins *in vivo.*** Journal of American Science 2011;7(1):772-783]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words**, Mycotoxin, Aflatoxin, Peanut, Toxicity, Probiotic bacteria		
81	Journal of American Science 2011;7(1):784-790]. (ISSN: 1545-1003). http://www.americanscience.org. 7	Full Text	81
82	**Bitopological spaces via Double topological spaces** A. KANDIL$^{\perp}$ O. TANTAWY* S.A.El-Sheikh** M. WAFAIE*** $^{\perp}$Mathematics Department, Faculty of science, Helwan University, P.O.Box 11795, Cairo, Egypt. * Mathematics Department, Faculty of science, Zagazig University, Egypt. ** Mathematics Department, Faculty of Education, Ain Shams University, Egypt. *** Modern Academy, For Engineering &Technology In Maadi, Egypt. dr.ali_kandil@yahoo.com **Abstract:** In this paper we shall study some bitopological properties via double topological spaces. We characterize the notions of pairwise continuous (resp. pairwise open, pairwise closed)(P .continuous, P -open, P -closed, for short) by a double continuous (resp. double open, double closed) mappings between double topological spaces. Also, we characterize the notions of P^{*} - continuous (resp. P^{*} -open, P^{*} -closed) by a supra double continuous (resp. open, closed) mappings between supra double topological spaces. Finally, we investigate the relationships between these types of mappings and give some counter examples. **[**A. KANDIL O. TANTAWY S.A.El-Sheikh M. WAFAIE. **Bitopological spaces via Double topological spaces.** Journal of American Science 2011;7(1):791-798]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** bitopological spaces, pairwise continuous mappings, supra- topological spaces, pairwise open mappings, pairwise closed mappings	Full Text	82
83	**Synthesis and some applications of Anionic Palmitic Acid Schiff Base Salt Surfactants** Aiad, I., Ahmed, S. M. and Dardir . M. M* Egyptian Petroleum Research Institute, Cairo, Egypt. monamdardir@yahoo.com * **Abstract:** Schiff bases derived from condensation reaction of benzaldehyde or anizaldehyde and diethylenetriamine were prepared. The products were reacted with palmatic acid (1 : 1 *mol) to give the corresponding palmitic Schiff base salt surfactants . The chemical* structures of the prepared compounds were confirmed using elemental analysis, FTIR and ^{1}H-NMR spectroscopy. Various surface properties of the synthesized surfactants were evaluated particularly, critical micelle concentration, effectiveness, efficiency, maximum surface excess and minimum surface area . These surfactants were also evaluated as corrosion inhibitors and as biocide agents Gram positive and Gram negative bacterial strains. The rheological properties, and the filter loss for oil-based mud (invert - emulsion mud) were evaluated, the result showed that they were a good emulsifiers and filter loss control agent for oil – base mud. It has been found that they have good corrosion inhabitation for low carbon steel alloy and has good bactericidal effect. [Aiad, I., Ahmed, S. M. and Dardir. M. M. **Synthesis and some applications of Anionic Palmitic Acid Schiff Base Salt Surfactants.** Journal of American Science 2011;7(1):799-807]. (ISSN: 1545-1003). http://www.americanscience.org.	Full Text	83

	Key words: Surfactants, Corrosion inhibitors, oil base mud and biological activity		
84	**Occupational Health Hazard of Egyptian Employees in Contact with Wastage Nourished Swine** Ashraf, M. Barakat[*1]; Hassan, A. El Fadaly[1]; Raafat, M. Shaapan[1] and Fathia, A.M. Khalil[2] [1]Zoonotic Diseases Department, National Research Center, Giza, Egypt [2]Parasitology and Disease Department, National Research Center, Giza, Egypt ashrafbarakat2@hotmail.com [*] **Abstract:** Egyptian swine still they are free nourished on wastages in small herds without veterinary health measures. Because of their omnivore's behavior, pigs are naturally exposed to zoonotic agents in their setting with subsequent direct human occupational hazards. Brucellosis, Leptospirosis and Toxoplasmosis are the major diseases link human exposure for natives in contact with swine. So, updating the sero-prevalence of these pathogens among contact employees reflect to how extent the human bio-hazards are due to direct contact with swine or their contaminant subset. Therefore, sera of 230 free wastage nourished pigs were collected at Cairo, Egypt. Also, 127 serum samples were collected from racing occupational workers. Human and swine sera were serologically analyzed for antibodies against Brucella, Leptospira and Toxoplasma by using commercial kits. Antibodies against *Brucella* were detected in 29/ 230 (12.61 %) of swine sera, and 11/127 (8.66 %) of workers sera by using Rose Bengal plate test. Antibodies against *Leptospira* serovars were detected in 53/230 (23.04%) of swine sera using the microscopic agglutination test (MAT) at a titer of 1:200. The highest seroprevalence was recorded for *L. pomona* (45.28%), followed by *L. grippotyphosa* (33.96%) and *L. icterohaemorrahgiae* (20.75%). The seropositive human sera were 25.9% with the highest incidence corresponding to *L. pomona* serovar (11%). Results of the indirect fluorescent antibody test showed that anti-*Toxoplasma* antibodies were detected in 74.78% (172/230) and 37.79% (48/127) of swine and contact employees respectively. It can be concluded that serological assays concerning brucellosis, leptospirosis and toxoplasmosis verify direct occupational exposure for high risk group's manipulating employees through carrier animals or their pollutant conditions. **[**Ashraf, M. Barakat; Hassan, A. El Fadaly; Raafat, M. Shaapan and Fathia, A.M. Khalil. **Occupational Health Hazard of Egyptian Employees in Contact with Wastage Nourished Swine.** Journal of American Science 2011;7(1):808-903]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words:** health hazard, swine, Brucellosis, Leptospirosis, Toxoplasmosis, Egyptian employees	Full Text	84
85	**Study of medical plant distribution in Lasem area of Northern Iran** Abed Vahedi[1], Esmaeil Yasari[2] [1]Corresponding author: Department of Agronomy and Plant Breeding, Faculty of Agricultural and Natural Resources, Islamic Azad University, Qaemshahr Branch, Qaemshahr, Mazandaran, 48148-35497. Cell: +98-09356211306. Iran. abedvahedy@gmail.com [2]Assistant Prof, Payame Noor University, Sari, Mazandaran, 48189-35455. Cell: +98-9113511510, Iran. e_yassari@yahoo.com **Abstract:** In order to gather and identify the medicinal plants at the mountainous rangelands of Lasem in Larijan of northern Iran, the field survey method was done. The results showed that there were 42 medicinal species in the area belonging to 18 classes. The classes Rosaceae with 8, Compositae with 8, and Labiateae with 7 species had the biggest number of medicinal species; and the growth forms hemicryptophyte and trophyte were the most common. Furthermore, leaves and flowers were the main plant parts used, essence and tannin were the most common compounds, and the most common curative effect was as diuretic. The types, features, and the compounds found in the medicinal plants of this ecosystem suggest that this region has a high potential with regard to the production of medicinal plants; and if the exploiters of the rangelands get to know this potential, they will be able to maintain the ecosystem, to keep it sustainable, and to reap huge economic benefits as well. [Abed Vahedi, Esmaeil Yasari. **Study of medical plant distribution in Lasem area of Northern Iran.** Journal of American Science 2011;7(1):904-911]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words:** Chemical compounds, Curative effects, Lasem, Medicinal plants	Full Text	85
86	**Protective Effect of Taurine and Bismuth Subnitrate against Cyclosporine and NSAID-induced Nephrotoxicity in Rats**.	Full Text	86

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Abstract: The immunosuppressive drug cyclosporine (CSA) has been successfully used in several diseases with immunological basis and in transplant patients. Nephrotoxicity is the major limitation for CSA use. Recent evidence suggests that reactive oxygen species (ROS) play an important role in mediating CSA-induced nephrotoxicity. Co-administration of CSA and non steroidal anti-inflammatory drug (NSAID), sodium diclofenac (SD), increases the efficacy for pain relief in patients with rheumatoid arthritis. However, clinical studies showed enhancement of cyclosporine nephrotoxicity. To characterize biochemical parameters of nephrotoxicity, the study assessed the effect of CSA (10 mg/kg B.wt) alone or in combination with SD (10 mg/kg B.wt) for 6 weeks on serum creatinine (S.Cr), blood urea (BU), alkaline phosphatase (ALP), total protein (TP), albumin and gamma glutamyl transferase (GGT). Oxidative stress was also evaluated; lipid peroxide measured as malondialdehyde (MDA), lactate dehydrogenase (LDH), as well as oxidized and reduced glutathione (GSSG and GSH) in serum of adult albino rats. CSA alone caused significant rise in BU and S.Cr, serum ALP and GGT, while reduction of serum TP and albumin was observed. In addition CSA also alternated oxidative stress through increasing levels of serum MDA, LDH and GSSG and decreasing levels of GSH and GSH/GSSG ratio. When SD combined with CSA, it enhanced all biochemical parameters of CSA-induced nephrotoxicity. The study also extended to evaluate and compare the protective effect of taurine, (tau), which is a major intracellular free beta-amino acid and potent endogenous antioxidant with Bismuth subnitrate (BSN), an antiulcer drug and a specific inducer of renal metalothionine (MT), against nephrotoxicity induced by CSA and SD administration. The present investigation showed that co-administration of both BSN and taurine could antagonize most of CSA negative effects, by attenuating renal dysfunctions, reducing serum MDA and counteracting the deleterious effects of CSA on oxidative stress markers.
[Suzan F.I. Elsisi, Salwa Kamal El –Nabarawy. **Protective Effect of Taurine and Bismuth Subnitrate against Cyclosporine and NSAID-induced Nephrotoxicity in Rats**. Journal of American Science 2011;7(1):912-921]. (ISSN: 1545-1003). http://www.americanscience.org.

Key Words: Nephrotoxicity, drug interaction, reactive oxygen species

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Protective Effect of Spirulina Against Mitomycin C-Induced Genotoxic Damage in male Rats

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Abstract: *Spirulina platensis* (SP) is a filamentous cyanobacterium microalgae with potent dietary phyto-antioxidant, anti-inflammatory and anti-cancerous properties. The present study aimed to investigate the protective effect of Spirulina against Mitomycin C (MMC)-Induced genotoxic damage in male rats. To evaluate the protective role of *Spirulina platensis* expression alterations of the Bcl-2, CK8, CK19, p53, p21, and p27 genes and formation of micronucleus in male rats were investigated. Sixty Swiss albino male rats were divided into six groups. Group 1, animals were fed on a standard diet as untreated control group. Group 2 animals were fed on a standard diet mixed with 1% SP. Groups 3, animals were fed on a standard diet mixed with 1% SP powder followed by MMC (0.5 mg/kg). Group 4 animals were fed on a standard diet mixed with 1% SP powder followed by MMC (2 mg/kg). Groups 5 and 6 animals were fed on a standard diet followed by MMC (0.5 and 2 mg/kg, respectively. All the animals were sacrificed after an experimental period of 12 weeks. The expression of Bcl-2, CK8, CK19, p53, p21 and p27 genes was investigated using reverse transcription polymerase chain reaction (RT-PCR). The results revealed that MMC treatment induced expression alterations of genes related to apoptosis. Also MnPCEs formation was increased in bone marrow of male rats treated with MMC. These alterations of the gene expression as well as the MnPCEs formation were markedly suppressed when male rats were supplemented with SP for 12

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	weeks. Conclusion: These findings suggest that SP exerts its anti-mutagenic properties by inhibiting alterations in the gene expression and the MnPCEs formation in the hepatic tissues and bone marrow cells of male rats exposed to MMC. [Sabah Abdulaziz Linjawi. **Protective Effect of Spirulina Against Mitomycin C-Induced Genotoxic Damage in male Rats**. Journal of American Science 2011;7(1):922-931]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** *Spirulina platensis*, Mitomycin C, Gene expression; RT-PCR; Rats; MnPCEs formation		
88	**Study of Sub-basal and Anterior Stromal Nerves of Corneal Flap with Modified Gold Chloride Stain** Sherif H Emerah MD, Hany M Labib MD, Ehab EL zakzouk MD, Ahmed A Zaki MD Cornea and ocular surface unit, Research Institute of Ophthalmology, Cairo, Egypt Corresponding author: ahmedazaki@hotmail.com **Abstract: The aim** was to study the regeneration of corneal nerve fibers following creation of corneal flap. **MATERIALS AND METHODS:** Nine white rabbits underwent creation of corneal flap only without the subsequent excimer laser photoablation, rabbits were scarified at 3 days, one week, two weeks and one month after the procedure. Demonstration of the corneal innervation was carried out with a modified gold chloride procedure. The tissue was dissected into 4-6 lamellae before dehydration and mounted on slides for observation and photography. **RESULTS:** At the 1st week, both superficial , basal epithelial and sub-epithelial nerves were found at the hinge of the flap but the rest of the flap showed a major loss of epithelial, basal subepithelial and superficial stromal nerves. At 1st month, A few new regenerating thin nerve fibres were found to emerge from the cut stromal nerve trunks. In addition,the anterior stromal nerve were thin with gradual restoration to its normal condition over time. At 6th month, The Sub-basal plexi returns to its pre-operative shape. The nerves of flap stroma become well developed. **CONCLUSION**: The number of sub-basal and stromal nerve fiber bundles almost completely disappeared after creation of flap. Sub-basal and anterior stromal nerves were still less than normal after 6 months. ***Key words*:** gold chloride, corneal nerves. [Sherif H Emerah, Hany M Labib, Ehab EL zakzouk, Ahmed A Zaki. **Study of Sub-basal and Anterior Stromal Nerves of Corneal Flap with Modified Gold Chloride Stain**. Journal of American Science 2011;7(1):932-936]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Sub-basal; Anterior; Stromal; Nerve; Corneal; Flap; Gold Chloride Stain	Full Text	88
89	**Simulation Optimization Approach for Facility Layout Problem-A Queuing Theory Based Approach** Seyed Mohammad Taghi Fatemi Ghomi, Amir Ardestani Jaafari Industrial Engineering department at Amirkabir University of Technology, Tehran, Iran. ardestani.amir@aut.ac.ir **Abstract:** One of the most important issues in facility layout problem is to find the location of the Input/ Output points. We consider single loop path as material flow path for a given layout and find locations of Input/Out points on perimeter of the loop in the uncertain environment. The uncertainty is derived from production time of each department. Our objective is to minimize total time of AGV system after conveying all departmental material flows, we solve an uncertain queuing problem and due to difficulty of the queuing problem, an efficient simulation optimization approach is proposed using simulated annealing algorithm. [Seyed Mohammad Taghi Fatemi Ghomi, Amir Ardestani Jaafari. Simulation Optimization Approach for Facility Layout Problem Using Queuing Theory. Journal of American Science 2011;7(1):937-941]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Facility layout; input/ output points location; queuing theory; simulated annealing	Full Text	89
90	**Sporicidal Effect of Ozone on Fungal and Bacterial Spores in Water Disinfection**	Full Text	90

	Roushdy M.M.[*], Abdel-Shakour E.H. and Abdel-Ghany T.M. Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt [*]m27roushdy@yahoo.com **Abstract:** The sporicidal effects of high ozone concentrations were tested against an endospore forming bacterial strain (*Bacillus subtilis* ATCC 6633) and a fungal strain (*Aspergillus brasiliensis* ATCC 16404) as a method of water disinfection. We compared the sporicidal action of ozone against these fungal and bacterial strains. Under identical treatment conditions, ozone showed a sporicidal effect on bacterial and fungal spores in water. Our present results showed that ozone concentrations at 7.0 and 9.0 g/m^3 have a sporicidal effect against bacterial and fungal spores respectively. Electron microscopic study of ozone-treated *B. subtilis* and *A. brasiliensis* spores mentioned above suggests the outer spore coat layers as a probable site of action of ozone. Our present study on ozone supports the notion that oxidizing agents including ozone probably kill spores by degrading the outer spore components and exposing the spore core to the action of the sanitizer. The ozone was generated using coaxial dielectric-barrier-discharge (DBD) technique. The coaxial DBD cell consists of two cylindrical coaxial electrodes separated by a gap distance and dielectric barrier (glass). AC (50 Hz) high voltage (2-5 kV) was applied on the DBD cell to generate filamentary discharge. The DBD cell is fed by oxygen gas. The basic mechanism of ozone generation simply consists of dissociation of oxygen molecules by the discharge electrons that are formed in the discharge filaments inside the discharge gap. The atomic oxygen, which is produced due to the dissociation, reacts with the oxygen molecules to form ozone. In the discharge, the oxygen molecules are dissociated prior to ozone formation. The concentration of the generated ozone was controlled by the discharge current and the gas flow rate. The generated ozone was used to treat the spores under investigation. [Roushdy M.M., Abdel-Shakour E.H. and Abdel-Ghany T.M. Sporicidal Effect of Ozone on Fungal and Bacterial Spores in Water Disinfection. Journal of American Science 2011;7(1):942-948]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Sporicidal; Fungal; Bacterial; Ozone; water; Disinfection		
91	**Diversity of Medicinal Plants in the Biospherical Reservation Areas of Iran (A Case Study of the protected area of Miankaleh)** Abed Vahedi[1] , Esmaeil Yasari[2] [1]Corresponding author: Department of Agronomy and Plant Breeding, Faculty of Agricultural and Natural Resources, Islamic Azad University, Qaemshahr Branch, Qaemshahr, Mazandaran, 48148-35497. Cell: +98-09356211306. Iran. abedvahedy@gmail.com [2]Assistant Prof, Payame Noor University, Sari, Mazandaran, 48189-35455. Cell: +98-9113511510, Iran. e_yassari@yahoo.com **Abstract:** Awareness of people concerning the side effects of chemical drugs has caused an increasing interest in traditional medicine. This study was carried out to gather and identify medicinal plants, their curative effects and the part of them which is used from the reservation area of Miankaleh. The region under study has an area of 68800 hectares situated 12 kilometers north of the city of Behshahr and northwest of the city of Gorgan. During numerous visits to the area, plants were gathered and, after their identification using specialized references of medicinal plants, the part used and the curative effects of the plants were determined. Results obtained showed that out of a total of 43 families, 125 genera, and 155 species found in the region, 33 families, 52 genera, and 61 species (39% of all the species) belonged to medicinal plants, among which the class Asteraceae with 6 species and the class Chenopodiaceae with 5 species had the most medicinal species. The most used parts of the plants were the leaves with 31%, the whole plants with 19%, and the roots with 15%. [Abed Vahedi, Esmaeil Yasari. Diversity of Medicinal Plants in the Biospherical Reservation Areas of Iran (A Case Study of the protected area of Miankaleh) Journal of American Science 2011; 7(1):949-953]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words:** Miankaleh, Medicinal plants, Boispherical reservation area, traditional medicine	Full Text	91
92	**Comparative Study and Feed Evaluation of Sprouted Barley Grains on Rice Straw Versus Tamarix Mannifera on Performance of Growing Barki Lambs in Sinai** Afaf M. Fayed	Full Text	92

	Animal and Poultry Nutrition Department, Desert Research Center, Mataria, Cairo, Egypt a_fayed2007@yahoo.com **Abstract:** In arid and semi arid areas Tamarix mannifera (Tm) was considered one of the principal feed resources , rice straw (Rs) one of agriculture wastes produced in a large amount but they have low nutritive value so several treatments were applied to ameliorate the utilization of Tamarix and rice straw. The objective of this study was to investigate the effect of sprouted barley on Tm , Rs and mixture of them. Thirty five growing femal Barki lambs of about four months old with an average live body weight (L.B.W) of 16 $\pm$ 0.5kg were divided into five treatments (7 animals each) to receive one of the following experimental roughages: treatment T_1 : rice straw (Rs) ad-lib (untreated) as control; T_2:dried Tamarix ad-lib(Tm)as control ;T_3 : sprouted barley grains on rice straw ad-lib (BRs) ; T_4 : sprouted barley grains on driedTamarix ad- lib (BTm) ; T_5 : sprouted barley grains on 50 % Rs + 50 % Tm ad-lib (BRs+ BTm) . The experimental growing trial lasted for about 180 day. All animal treatments were fed 60% of total energy requirement as concentrate feed mixture (CFM). At the end of the growing trial five digestibility trial were conducted to evaluate the digestibility of the experimental roughages. Results showed that the treatments with sprouted barely increased CP, Ash and NFE while DM, OM, EE, CF, NDF, ADF and ADL contents, were decreased. Sprouted barely on Tamarix (BTm) or rice straw (BRs) revealed a significant ($P \leq 0.05$) improvement in OM, CP, EE and cellulose digestibility with an insignificant higher in CF, NDF and hemicellulose digestibility. Nutritive values expressed as TDNg/Kg B.W. and DCP% increased significantly ($P \leq 0.05$) with treatments T_2, T_3 and T_4 than untreated T_1 (Rs) and T_5 (Tm). Also, ewes fed the treated roughages retained higher ($P \leq 0.05$) nitrogen values than untreated treatments. Ewes fed sprouted barely had significantly higher ($P \leq 0.05$) values of total volatile fatty acids (VFA), ruminal ammonia (NH3- N) concentration, serum total proteins. Albumin and urea, was insignificantly increased, while serum globulin and creatinin were insignificantly decreased GOT, GPT activity than untreated roughages. The highest ($P \leq 0.05$) value of average daily gain , feed conversion (g feed/ g gain) and economical feed efficiency were recorded for T_4. However the lowest ($P \leq 0.05$) values were recorded for T_1. In conculusion we can produce green fodder by utilizing dried Tamarix and rice straw by simple methodology using crop sprouts (barley). [Afaf M. Fayed. **Comparative Study and Feed Evaluation of Sprouted Barley Grains on Rice Straw Versus Tamarix Mannifera on Performance of Growing Barki Lambs in Sinai**. Journal of American Science 2011; 7(1):954-961]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words**: Tamarix , rice straw , sprouted barley , sheep ,growth , rumen and blood parameters		
93	**Study of the Right Liver Lobe Size /Albumin Ratio as a Noninvasive Predictor of Oesophageal Varices Compared to: Spleen Size, Platelet Count and Platelet Count/Spleen Diameter Ratio in Post Hepatitis C Virus Liver Cirrhosis in Egypt** Serag Esmat[1]and Dalia Omran[2] [1]Department of Internal Medicine, Faculty of Medicine, Cairo University. [2]Department of Tropical Medicine, Faculty of Medicine, Cairo University seragesmat@hotmail.com **Abstract:** Back ground and aim: Hepatitis C Virus (HCV) is considered the most common aetiology of chronic liver disease in Egypt.Portal hypertension is a major complication of liver cirrhosis, and leads to the development of portosystmic shunts. Oesophageal varices are the most important among these shunts. Bleeding from oesophageal varices is the most serious complication of cirrhosis, with a high risk of death. The prevention of variceal bleeding is very important, non-selective beta blockers and prophylactic band ligation decrease the risk of bleeding by 50%. The current guide lines recommend screening of all cirrhotic patients by endoscopy, to identify patients at risk of bleeding so prophylactic treatment should be started to them. But repeated endoscopic examinations are unpleasant for patients, and carries high cost impact and more burden on endoscopic units, while only 50% of cirrhotic patients have esophageal varices, and up to 30% have large varices. For these reasons many non-invasive predictors for the presence and size of varices have been studied. The aim of this study is to evaluate prospectively the right liver lobe size /albumin ratio and to compare it with spleen size, platelet count and platelet count/spleen diameter ratio as noninvasive predictors of oesophageal varices in post hepatitis C virus liver Cirrhosis in Egypt. Patients	Full Text	93

	and methods: This prospective study included one hundred patients with post hepatitis C virus liver Cirrhosis. All studied subjects underwent a detailed history taking, clinical examination and a biochemical workup, including total bilirubin, aspartate aminotransferase, alanine aminotransferase, serum albumin, prothrombin activity, complete blood count and viral markers for hepatitis C and hepatitis B viruses. Child-Pugh score was calculated for all patients. An upper gastrointestinal endoscopy and abdominal ultrasound were performed for all patients. The platelet count to spleen diameter ratio and the right liver lobe to albumin ratio were calculated. Results: All the 4 predictors showed high statistically significant correlation with the presence and the grade of oesophageal varices (P values <0.001) Among the 4 noninvasive predictors the platelet count/spleen diameter ratio gave the highest accuracy at a cut-off value of 1326.58 (sensitivity 96.34% and specificity 83.33%) followed by the right liver lobe/albumin concentration ratio at a cut-off value of 44.2 (sensitivity 91.46% and specificity 77.78%) followed by the spleen size at a cut-off value of 131.5mm(sensitivity 90.24% and specificity 83.33%) then lastly the platelet count at a cut-of value of 131000/mm^3(sensitivity 84.15% and specificity 83.33%). [Serag Esmat and Dalia Omran. **Study of the Right Liver Lobe Size /Albumin Ratio as a Noninvasive Predictor of Oesophageal Varices Compared to: Spleen Size, Platelet Count and Platelet Count/Spleen Diameter Ratio in Post Hepatitis C Virus Liver Cirrhosis in Egypt**. Journal of American Science 2011; 7(1):962-968]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words:** Noninvasive predictors of oesophageal varices, the right liver lobe/albumin ratio, the platelet count/spleen diameter ratio, Oesophageal varices, Post HCV liver cirrhosis		
94	**Strategies of Rural Development in Shoushtar Township of Iran (Applying SWOT method)** Ahmad Reza Ommani Assistant Professor Islamic Azad University Shoushtar Branch, Iran Ommani@ijamad.com **Abstract:** The purpose of this research was using SWOT for identifying strategies of rural development in Shoushtar township of Iran. SWOT technique used for clarifies strengths, weaknesses, opportunities, and threats of rural area in Shouahtar Township, Iran. The population of study was people of rural area of Shoushtar. The sample size (n=110) determined by Cochran formula and selected by random sampling. Based on the results, external (opportunities and threats) and internal (strengths and weaknesses) factors that affected on situation of rural area were evaluated. Based on the participant's idea, each item ranked and importance ratio coefficient identified. Based on the results the score of external and internal factor were 2.05 and 1.71. Also, SWOT results indicated important strategies for rural development were: SO_1: Using new technology for increasing productivity, SO_2: Planting new crops with high economic value, ST_1: Designing developmental plan for development markets, ST_2: Environmental and natural sustainability, ST_3:Development of agricultural policy regarding efficiency use of possibilities, WO_1: Using new technology for public services, WO_2: Development of extension program for HRD, WT_1: Development practices for contracting equality in social and economical condition and WT_2: Development of agricultural policies for productivity in poor farmers practices. **[**Ahmad Reza Ommani. **Strategies of Rural Development in Shoushtar Township of Iran (Applying SWOT method)**. Journal of American Science 2011; 7(1):969-972]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** SWOT, External Factor Evaluation, Internal Factor Evaluation	Full Text	94
95	**Serum Levels of cytokines in poly-transfused patients with Beta-Thalassemia major: Relationship to splenectomy** Mohga Shfik[1], Hayat Sherada[1], Yehia Shaker[2], Mie Afify[2], Howayda Ali Sobeh[3]and Samar Moustafa[2] [1.] Biochemistry - Division- Faculty of Science- Helwan University [2.] Biochemistry Department- National Research Centre- Dokky- Egypt [3.] New Paediatric Hospital- Haematology Department- Faculty of Medicine- Cairo University **ymshaker@yahoo.com** **Abstract :** Beta thalassemia is the most common chronic haemolytic anemia in Egypt. A major cause of morbidity and mortality in β-thalassemic patients is infections, assumed to be the result of immunological	Full Text	95

	changes. Cytokines production by immune cells is superior representative of phenotypes and functions of lymphocytes, but results of previous researches are not satisfactory and in some cases are controversial, due to differences in their experimental designs. So the aim of this study was to determine the possible defect, we investigated the cytokine IL-2 and IL-8 productions by blood cells of β-thalassemic patients. The study was conducted on fifty one patients with homozygous beta-thalassemia major (23 of them were splenectomized group 1), who attending the Haematology Clinic, New Paediatric Hospital, Faculty of Medicine, Cairo University. Beside 17 healthy subjects served as control, with the same age matched group. All subjects were subjected to: full clinical examination, complete blood counting, liver function tests, and renal function tests. Determination of IL-2 was done by an immunoenzymometric assay for the quantitative measurement (Biosource IL-2 EASIA kit), and Determination of IL-8 by AviBion Human Interleukin-8 ELISA kits. The result showed that, there were significant increase **(P** <0 .05) in the serum level of IL-8 among group 1(mean level was 526.4 ± 65.7 U/ml) as compared to control group (mean level was 208.67 ± 35.53 pg/ml) as well as group 2 (mean level was 438.21 ± 58.063 pg/ml). Also group 2 had significant increase **(P** <0 .05) in the serum level of IL-8 as compared to control group. While, the levels of serum IL-2 showed no significant changes **(P** >0 .05) between the thalassaemic groups as well as the control group. In conclusion, the study revealed that beta-thalassemia major patients had increased level of IL-8 which was more prominent in splenectomized patients. The potential role of IL-8 and the interactions between different cytokines in thalassaemic patients require further investigation. Multi-transfusions could be responsible for a change in circulating cytokines that could contribute to a state of partial immune deficiency in beta-thalassaemic patients, which is more prominence among the splenectomized patient. [Mohga Shfik, Hayat Sherada, Yehia Shaker, Mie Afify, Howayda Ali Sobeh and Samar Moustafa Serum Levels of cytokines in poly-transfused patients with Beta-Thalassemia major: Relationship to splenectomy. Journal of American Science 2011; 7(1):973-979]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words:** thalassemia major- IL-2, IL-8- splenectomy		
96	**Strategies for Retaining Youth in Rural Communities** Ahmad Reza Ommani Assistant Professor, Islamic Azad University-Shoushtar Branch, Khouzestan, Iran ommani75451@yahoo.com **Abstract:** The identify factors affecting on migration youths to urban centers is very important to rural program development. For develop strategies that attract and keep youth in rural communities, reasons youth migrate to urban centers must be closely examined and identified. The research method employed was correlative-descriptive. The population consisted rural youth in Shoushtar township of Khuzestan province in Iran. A random sample of rural youths (n=360) was selected. Data collected were analyzed using the Statistical Package for the Social Sciences (SPSS). Appropriate statistical procedures for description (frequencies, percent, means, and standard deviations) were used. The main result of the study revealed that top reasons by youth for moving to urban centre including: employment, education, family-related and to get away. Also the top eight strategies for retaining youth to rural communities were: Improve career opportunities**,** Provide work experience opportunities **,** Improve opportunities for education after high school**,** Improve opportunities for social activities**,** Improve access to amenities**,** Promote the advantages of rural living**,** establishment of youth advisory committees establishment of youth priorities for local government**,** Promote youth involvement in community decision making. From a development perspective, the youth are the future for any country and the world. The potential of youth to transform rural communities needs to be recognized, especially in developing countries where the majority of citizens depend on agriculture as a source of livelihood. If rural development is to be sustainable, the rural youth need to be brought in the mainstream of the development process, no matter whether the development initiatives come from the public or private sector. Rural development in the long-term depends on how the youth are prepared to cope with the challenges they are likely to face as rural citizens. [Ahmad Reza Ommani. Strategies for Retaining Youth in Rural Communities. Journal of American Science 2011; 7(1):980-983]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Youth, Rural development, Employment, Migration	Full Text	96
97	**Melatonin Supplementation Could Trigger Delayed Cardiac Preconditioning Against I/R Injury in Partial Nephrectomized Rats with Emphasis to Possible Role of Cardiac NO.**	Full Text	97

Bataa M.A .El –Kafoury1*, Amira M. Abdel- Rahman1and Fayda I. Abdel Motaleb2
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Abstract: The cardioprotective effects of melatonin are consistent with its ability to scavenge free radical. However free radicals are considered as preconditioning factors, helping the heart to withstand consequent attacks of ischemic reperfusion injury. So, this study aimed to clarify whether melatonin supplementation, concomitant with the deterioration of kidney function in experimental model of renal failure, is able to protect the isolated heart against the liability for global ischemic reperfusion (I/R) injury or its antioxidant effect interferes with proposed preconditioning effect of free radical. Moreover, the study evaluated the changes of myocardial nitric oxide (NO) system with melatonin treatment as one of the suggested triggers of preconditioning. Thirty male Albino rats were divided into three equal groups, sham- operated control rats, 5/6 subtotal nephrectomized (STNx) group and 5/6 subtotal nephrectomized melatonin- supplemented (STNx + M) group. Melatonin was given at a dose of 5 mg / kg/ day for 8 weeks. Rats in all groups were subjected to estimation of plasma urea, creatinine, malondialdehyde (MDA) and nitrate levels, followed by perfusion of isolated hearts. A period of ischemia (30 min) followed by reperfusion for another 30 min was done. The cardiac hemodynamic changes during reperfusion at 5, 15, 25 and 30 min intervals were recorded. At the end of reperfusion, the different chambers of the heart were subjected for determination of the absolute weights as well as their weights to body weight ratios. Sections from the cardiac muscle, mainly ventricle, were used for tissue reduced glutathione (GSH) and nitrate estimation. Partial nephrectomized group (STNx) exhibited significant deterioration of the baseline cardiac hemodynamic as well as more liability for ischemic reperfusion injury in early (5 min) and late reperfusion (30 min) records. Also nephrectomy caused significant cardiac remodeling (hypertrophy), manifested in the increased left ventricle and whole cardiac weights to body weight ratio. The significantly increased plasma MDA, urea and creatinine with nephrectomy showed a negative correlation with the reduced plasma and cardiac tissue nitrate. Melatonin treatment concomitant with the deterioration of renal function(in STNx +M group) showed significant higher basal coronary flow compared to STNx group but it did not improve the ameliorate basal intrinsic cardiac activity due to renal failure. Following I/R, melatonin pre treated group showed some sort of protection against deterioration of cardiac activity in particular at 30 min reperfusion. A 44.5 % decrease in HR in STNx rats versus 30.5% decrease in HR in melatonin treated has been observed. Also the percentage of decrease in peak tension and the tension /left ventricular weight due to reperfusion were significantly lower with melatonin treatment at both 5 and 30 min records of reperfusion. Also melatonin shortened the time to peak tension (TPT) in particular at 30 min reperfusion where ,the increase in TPT due to reperfusion injury was +20.3% with melatonin treatment versus +51.9% in non treated rats. Although, melatonin shortened the half relaxation time(1/2RT) and improve the myocardial flow rate(MFR) compared to non treated group in some records of reperfusion but compared to basal record ; the percentage of change was non significant. Melatonin significantly decreased urea, creatinine and MDA levels which still higher compared to sham control group. Also melatonin ameliorated the hypertrophic changes but not completely with an increase in cardiac tissue GSH and nitrate levels in hearts of melatonin treated rats as well as plasma nitrate. The increased MDA which is an indicator for free radical generation in partial nephrectomized rats did not provide the supposed preconditioning effect against ischemic reperfusion injury in isolated hearts or its effect wasn't conclusive. On the other hand, melatonin was able to improve the basal coronary flow rate and appears to offer some sort of preconditioning and/or protection against I/R injury a condition of excess free radical generation. Cardiac tissue GSH (anti-oxidant) and NO triggering by melatonin may be added to its free radical scavenging effect in the suggested protection and / or preconditioning.

[Bataa M.A .El –Kafoury, Amira M. Abdel- Rahman and Fayda I. Abdel Motaleb. **Melatonin Supplementation Could Trigger Delayed Cardiac Preconditioning Against I/R Injury in Partial Nephrectomized Rats with Emphasis to Possible Role of Cardiac NO.** Journal of American Science 2011; 7(1):984-998]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: cardiac preconditioning, ischemic reperfusion, melatonin, nitric oxide, free radicals, partial nephrectomy

98	**Evaluation of Serum Chromogranin A as a Useful Tumor Marker for Diagnosis of Hepatocellular**	Full Text	98

	Carcinoma Ahmed M. Awadallah[*1], Hesham Ali Issa[1] and Mohamed S. Soliman[2] Department of Clinical and Chemical Pathology[1] and Department of Hepatology, Gastroenterology and Infectious diseases[2], Faculty of Medicine, Benha University, Benha, Egypt. [*]a_mamdouh8@hotmail.com **Abstract:** Background: In Egypt, HCC was reported to account for about 4.7% of chronic liver disease patients. Approximately 80% of HCCs are associated with cirrhosis, which is regarded as the most important precancerous etiological factor. Chromogranin A is a cellular marker for neuroendocrine tumors. High serum levels of CgA have also been demonstrated in patients with other malignancies including colon, lung, breast and prostate cancer. Objective: To evaluate serum CgA as a marker for HCC. Patients and Methods: Eighty cases (30 with HCC, 30 with liver cirrhosis and 20 apparently healthy controls) were subjected for estimation of Chromogranin A (CgA) and Alpha feto protein (AFP) by ELISA technique together with routine laboratory investigations including CBC, prothrombin time and concentration and INR and serum urea, creatinine, albumin, AST, ALT, alkaline phosphatase and bilirubin (total and direct). Results: There was a highly significant statistical difference between control group and HCC group and between liver cirrhosis group and HCC group as regard to AFP and Chromogrnin A ($P<0.01$). There was a significant statistical difference between control group and liver cirrhosis group as regard to AFP and Chromogrnin A ($P<0.05$). Conclusion: the results of the present study revealed that the application of CgA as a tumor marker in the diagnosis of HCC is to be considered especially in cases with low levels of AFP, as determination of CgA serum values represents a complementary diagnostic tool in monitoring chronic liver disease patients for detection of HCC. The combined use of both CgA and AFP to detect HCC increases their sensitivity and specificity. [Ahmed M. Awadallah, Hesham Ali Issa and Mohamed S. Soliman. **Evaluation of Serum Chromogranin A as a Useful Tumor Marker for Diagnosis of Hepatocellular Carcinoma**. Journal of American Science 2011; 7(1):999-1007]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Chromogranin A, Hepatocellular carcinoma, liver cirrhosis		
99	**Monte Carlo method and the Ising model for magnetized and non-magnetized water as MRI contrast agent** Wael Abou EL-wafa. Ahmed [1], Yasser M. Kadah [2], Samir M. Badawi [3] [1.] Biomedical Engineering Department, Faculty of Engineering, Minia University, Egypt [2] Biomedical Engineering Department, Faculty of Engineering, Cairo University, Cairo, Egypt [3] Industrial Electronics and Control engineering, Faculty of Electronic Engineering, Monoufia University, Egypt wael@eng.miniauniv.edu.eg **Abstract:** A Monte Carlo algorithm for a two dimensional Ising model is proposed and implemented using Mat lab. It describes a lattice with a discrete number of particles. We study the evolution of the system over time depending on a particular variable called the interaction strength .The results of computer simulations agree with practical experiments showing that there is a change in Energy-Magnetization and strength interaction-Magnetization curves between magnetized water and normal water which means that the magnetized water or Saline changes the properties of the solutions affecting T1 so it can be used as a new contrast agents for MRI. [Wael Abou EL-wafa. Ahmed, Yasser M. Kadah, Samir M. Badawi. **Monte Carlo method and the Ising model for magnetized and non-magnetized water as MRI contrast agent**. Journal of American Science 2011; 7(1):1008-1012]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords**: Monte Carlo; MRI; magnetized water; Ising.	Full Text	99
100	**Multidrug resistant Egyptian isolates of *Acinetobacter baumannii*** [1] Shabaan Hashem Ahmed; [2]Sayed Fekry Abdelwahab; [3] Ayman Mohammed Hasanen; [4]Doaa Safwat Mohammed[*] [1]Department of Microbiology and Immunology, Faculty of Pharmacy, University of Assuit, Egypt. [2] Department of Microbiology, Faculty of Medicine, University of Minia, Egypt. [3] Department of General Surgery, Faculty of Medicine, University of Minia, Egypt.	Full Text	100

	[4]Department of Microbiology, Faculty of Pharmacy, University of Beni-Suef, Egypt. doaa.safwat@yahoo.com **Abstract:** The resistance of *Acinetobacter baumannii* to antimicrobial agents is mediated by all of the major resistance mechanisms, including modification of target sites, enzymatic inactivation and active efflux of drugs. Antibiotic susceptibility testing has been performed on fifty-two *A. baumannii* isolates. Twenty isolates have been recovered from patients suffering from wound and burn wound infections attending general surgery, plastic surgery and obstetrics and gynecology departments and thirty-two isolates have been recovered from the environment of these departments. Different mechanisms of antimicrobial resistance have been detected among resistant isolates. Broth dilution method have been used to investigate antimicrobial susceptibility pattern, iodometric method have been used to detect β-lactamase enzymes and polymerase chain reaction has been used to detect $bla_{oxa\text{-}51\text{-}like}$ genes, *aph (3)-VIa* genes and *adeB* gene. Tetracycline was the most effective antimicrobial agent against *A. baumannii*. It has showed high resistance to both of amikacin and meropenem (76.9%), cefipime (80.8%) and both of cephradine and imipenem (96.2%). An extreme resistance to the other antimicrobial agents has been shown by the same organism. β-lactamase enzyme has been detected in β-lactam resistant isolates, $bla_{oxa\text{-}51\text{-}like}$ carbapenemase genes have been detected in carbapenem resistant isolates, *aph (3)-VIa* genes have been detected in amikacin resistant isolates and *adeB* gene have been detected in some multidrug resistant strains. So, resistance to β-lactams, carbapenems and amikacin has been high in *A. baumannii* isolates which has caused appearance of multidrug resistant isolates with different resistance mechanisms like $bla_{oxa\text{-}51\text{-}like}$ genes, *aph (3')-VIa* genes and *adeB* gene. [Shabaan Hashem Ahmed; Sayed Fekry Abdelwahab; Ayman Mohammed Hasanen; Doaa Safwat Mohammed. **Multidrug resistant Egyptian isolates of *Acinetobacter baumannii*.** Journal of American Science 2011; 7(1):1013-1019]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** *A. baumannii*, $bla_{oxa\text{-}51\text{-}like}$ genes, *aph (3')-VIa* genes, *adeB* gene		
101	**Evaluation of the effect of three different pesticides on *Azolla pinnata* growth and NPK uptake** El-Shahate, R.M.[1] – El-Araby, M.M.I.[2] - Eweda, E.W[3] –El -Berashi, M.N.[2] 1. Soil, Water and Environ. Res. Inst., ARC, 2. Faculty of Science, Ain Shams University, 3. Faculty of Agriculture, Ain Shams University **Abstract:** Three pesticides of common use in rice fields in Egypt were used in the present work. This study was devoted to investigate the effects of different concentrations of the insecticide furadan, fungicide hinosan and herbicide saturn on the growth and NPK uptake of the aquatic fern *Azolla pinnata,* which is recommended to be applied as a biofertilizer in rice. In this respect, the results obtained showed variable effects of the three pesticides under study. Furadan and hinosan showed positive effects since each increased the growth rate of *A. pinnata* at lower concentrations (0.001, 0.002 ppm) and consequently increased its NPK content. Maximum dinitrogenase activity was also generally obtained at 0.002 ppm furadan, throughout the different incubation periods. Nitrogen, phosphorus and potassium uptake was generally increased with increasing the incubation period of the applied furadan and hinosan, at all concentrations. The highest NPK uptake by *A. pinnata* was obtained with the medium concentration (0.002 ppm) of both pesticides after 20 and 25 days of incubation. On the other hand, saturn generally showed inhibitory effects on the growth, N_2- fixation and NPK uptake even at lowest concentration (0.001 ppm). **[**El-Shahate, R.M. – El-Araby, M.M.I. - Eweda, E.W–El -Berashi, M.N. **Evaluation of the effect of three different pesticides on *Azolla pinnata* growth and NPK uptake.** Journal of American Science 2011; 7(1):1020-1031]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** *Azolla pinnata,* fungicides, insecticides, herbicides saturn, hinosan, furadan, growth, dinitrogenase activity, uptake of nitrogen, phosphorus, potassium.	Full Text	101
102	**An Analysis of Polyethylene Coating Corrosion in Oil and Gas Pipelines** Amir Samimi [a 1] ، Soroush Zarinabadi [2] [1.] Faculty member of Islamic Azad University, Mahshahr branch, Iran	Full Text	102

	[2.] Islamic Azad University, Mahshahr, Iran 1- amirsamimi1161@gmail.com 2- zarinabadi@yahoo.com **Abstract:** The corrosion of pipelines' coatings is one of the main problems in oil and gas industries for which a large amount of money is spent each year. Coating is the first defense line in front of a corrosive environment in which pipes have been buried. Good function of coating depends on its adhesiveness rate to the metal surface. Initial adhesiveness and its durability in the contact conditions are among those factors that enhance coating efficiency in long term. The rate of Initial adhesiveness has a high relationship with coating movement and surface wetness by this movement in the course of applying the coating and also with cleanliness and preparedness of pipe surface. The durability and permanence of adhesiveness depends on coating properties including its resistance in front of moisture penetration. Applying coating on the pipelines has a high cost so for this reason the selection and application of coating is of high importance. Also for underground buried pipes it is not possible to change their coatings in short durations unlike other structures. Therefore the coating must be durable for 20 years. This article proceeds to investigate the reason for corrosion in steel pipes with three poly ethylene layers. **[**Amir Samimi، Soroush Zarinabadi. **An Analysis of Polyethylene Coating Corrosion in Oil and Gas Pipelines.** Journal of American Science 2011; 7(1):1032-1036]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** corrosion ; initial adhesiveness ; poly ethylene coating		
103	**Scrutiny Water Penetration in Three-layer Polyethylene Coverage** Soroush Zarinabadi[*1] , Amir Samimi[2] 1- Faculty member of Islamic Azad University, Ahvaz Branch 2- Member of young researchers club, Islamic Azad University Mahshahr 1- zarinabadi@yahoo.com 2- amirsamimi1161@gmail.com **Abstract:** Coverage in line pipes include of high costs. For this selecting cover and how apply is high important. Three fold polyethylenes include of epoxy layers, adhesive and polyethylene. Each other from layers having attributes that increasing its application for long term. Polyethylene layer is good shelter for prevent of physical damages. In attention to corrosion in lower temperature is a electrochemical reaction and rate of a electrochemical reaction is very impress of a element or very reactor from surface. This position occurred when influence of a element increasing of other cover controllers. A example of this issue that will be cause of outer corrosion in pipes under soil and this is very importance in work, this is leakage water into covers that can be measurable with coefficient of water leakage that can exchanging layers quality. This article has studied leakage water into three fold polyethylene cover. **[**Soroush Zarinabadi, Amir Samimi. **Scrutiny Water Penetration in Three-layer Polyethylene Coverage.** Journal of American Science 2011; 7(1):1037-1039]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** leakage water; polyethylene cover; epoxy layer; outer corrosion	Full Text	103
104	**Effect of Feeding Different Sources of Energy on Performance of Goats Fed Saltbush in Sinai** Ahlam R.Abdou, E.Y. Eid ; Abeer M. El-Essawy, [*]Afaf M. Fayed, H.G. Helal and H.M. El-Shaer Department of Animal and Poultry Nutrition, Desert Research Center, Mataria, Cairo, Egypt [*]a_fayed2007@yahoo.com **Abstract:** Feeding halophytes is a feasible solution to minizme the problem of feed shortage in arid and semiarid areas of Egypt. This work aimed to investigate the effect of feeding goats on fresh *Atriplex nummularia* which is grown naturally and cultivated in Sinai on performance of growing goats when added with different sources of energy supplementation (concentrate feed mixture CFM, ground barley grains or ground date stones and mixture of these materials) on nutrients digestibility, nitrogen balance, water utilization and some rumen and blood metabolites. The experiment was performed on twenty eight of growing goats (six months old) with mean body weight 16 ± 0.38 Kg were divided into four equal groups for 105 days. The diets were given at the basis of 40:60 roughage: concentrate ratio for growth requirements. The roughages were berseem hay in T1 (control group) or fresh *Atriplex nummularia* in T2,	Full Text	104

	T3 and T4 whereas the energy supplements were concentrate feed mixture (CFM) in T1, ground date stones in T2, ground barley grains in T3 and a mixture of 50% ground barley grains with 50% ground date stones in T4. Results obtained revealed that inclusion of barley grains in T3 group improved DMI of Atriplex than that in T1, T2 and T4 groups. The highest body weight gain was recorded by animals in T1 and T3 compared to those of the other treatments. In addition Intakes of TDN and DCP were maximum in T1 and T3. The maximum apparent digestion coefficients of OM, CP, EE and NFE were recorded by animals in T3 while those of DM and CF were digested much better by animals in T1. TDN% and DCP% were increased in T1 followed by T4. All animals were in positive nitrogen balance. The maximum values of total water intakes were recorded for animlas in T2 whereas the lowest values for animals in T3 with significant differences. Serum creatinine, total protein, globulin and GPT levels were not affected by diet type and they were within the normal ranges. Also a sampling time factor was detected. Ruminal ammonia-nitrogen and total volatile fatty acids revealed significant variations before feeding and 6 hrs post feeding. The feed cost of daily gain (L.E)/ kg was achieved for animals fed ground date stone in T2 (L.E 0.860) which was lower than T4, T3 and T1 (L.E. 1.255, 1.273 and 1.290) respectively. In conclusion, barley grains or ground date stones or their mixture improved the nutrients utilization and intake of *Atriplex*. Utilization of such halophytic plants supplemented with non–conventional energy supplements could be recommended to enhance feed materials availability all-round year and to improve animal performance as well under arid and saline conditions of Sinai. [Ahlam R.Abdou, E.Y. Eid; Abeer M. El-Essawy, Afaf M. Fayed, H.G. Helal and H.M. El-Shaer. **Effect of Feeding Different Sources of Energy on Performance of Goats Fed Saltbush in Sinai.** Journal of American Science 2011; 7(1):1040-1050]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words:** Salinity, halophytes, saltbush, barley grains , date stone , goats, intake, nutrients digestion.		
105	**Factors Associated with the Distribution of the Invasive Bivalve Clams" *Donax Variabilis* (Say,1822)" at the Area of the Mediterranean Coast Preferred by Marine Fish Larvae, New Damietta, Egypt** El-Ghobashy, A.E.[1]; Mahmad, S.Z.[2]; Kandeel, S.K.[3] and El-Ghitany, A.H.[*1] [1]Zoology Department, Faculty of Science (Damietta), Mansoura University, Egypt. [2]Oceanography Department, Faculty of Science, Suez Canal University, Egypt. [3]Zoology Department, Faculty of Science (Fayoum), Fayoum University, Egypt. [*]asmaa_haris222@yahoo.com **Abstract:** New Damietta shore is one of the important areas for collection of the clams as well as mullet, seabass and seabream larvae which are reliable for marine aquaculture in Egypt. *Donax variabilis* was recorded for the first time in Egypt and because of its presence in the area of Damietta Maritime Port, larvae has come stuck with ships from the Atlantic Ocean where they were registered there. The density of *D.variabilis* increased in site I (718 / m^2) than in site II (415 / m^2). Water salinity (33.43 ± 4.59 mg/ L) in site I was less than the salinity of the sea, while it was almost similar to the salinity of the sea (36.94 ± 3.45 mg/ L) at site II. Nutrients concentration at site II were higher than that at site I, where it averaged 0.02 ± 0.01, 0.05 ± 0.03 and 0.26 ± 0.16 at site I and 0.05 ± 0.03, 0.34 ± 0.41 and 0.46 ± 0.36 mg/l at site II for NO_2, NO_3 and PO_4 respectively. Measured *Chlorophyll a* was high at site II (0.25 0.12 mg/m^3) compared to site I (0.25 0.12 mg/m^3), revealing the increase in phytoplankton biomass at site II. Crustaceans and molluscs were the most groups associated with clam's beds. *D.variabilis* cohorts appeared during summer months, this indicates that the population consists of only one spawning event. Length frequency of *D.variabilis* was essentially bimodal during the period of study. Three modes were recorded in June, 2008 at size classes of 7, 11and 20 mm of shell length. [El-Ghobashy, A.E.; Mahmad, S.Z.; Kandeel, S.K. and El-Ghitany, A.H. **Factors Associated with the Distribution of the Invasive Bivalve Clams" *Donax Variabilis* (Say,1822)" at the Area of the Mediterranean Coast Preferred by Marine Fish Larvae, New Damietta, Egypt.** Journal of American Science 2011; 7(1):1051-1062]. (ISSN: 1545-1003). http://www.americanscience.org. **Key wards:** Mediterranean coast- *Donax variabilis*- A biotic factors- Biotic factors	Full Text	105
106	**Antimicrobial Evaluation of Novel Pyrrole, Pyrazole, Pyrimidine and Pyrrolo [2, 3-d]-Pyrimidine Derivatives Brearing Sulfonamide Moiety**	Full Text	106

	Mostafa. M. Ghorab [1], Helmy. I. Heiba[2], Amina. A. Hassan[3], Amany. B. Abd El-Aziz [3], and Marwa. G. El-Gazzar[2*] [1] Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC), College of Pharmacy, King Saudi University, Riyadh, Saudi Arabia. [2] Department of Drug Radiation Research, National Center for Radiation Research and Technology, Cairo, Egypt. [3] Department of microbiology, National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt. *marwagalalgazzar@yahoo.com **Abstract:** Novel pyrrole **5** and **6**, pyrrolopyrimidine **7-10,** pyrazole **14** and **15** or pyrimidine **16** and **17** derivatives bearing biologically active sulfonamide moiety were synthesized and tested for their antimicrobial activity. The synthesized compounds possessed antibacterial and antifungal activities with MIC ranging from 4–256 µg/mL. The most resistant species was *Aspergillus flavus*, while the most sensitive were *Aspergillus fumigatus* and *Penicillium chrysogenum.* The results of the antimicrobial screening showed that all the tested compounds possess significant activity and some were found to be more active than the reference drugs used (ciprofloxacin and ciclopiroxolamine). **[**Mostafa. M. Ghorab, Helmy. I. Heiba, Amina. A. Hassan, Amany. B. Abd El-Aziz, and Marwa. G. El-Gazzar. **Antimicrobial Evaluation of Novel Pyrrole, Pyrazole, Pyrimidine and Pyrrolo [2, 3-d]-Pyrimidine Derivatives Brearing Sulfonamide Moiety.** Journal of American Science 2011; 7(1):1063-1073]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** antimicrobial, pyrrole, pyrazole, pyrimidine, pyrrolo [2, 3-d]-pyrimidine, sulfonamide		
107	**Egyptian Folk Art and its Significance as a Source of Symbolic Design Decorative Clothes Young Men and Women** [*]Rabab H. Mohammed and Sahar A. Zaghloul Department of Clothing and Textiles - College of Home Economics - Helwan University, Helwan, Egypt [*]rababh72@yahoo.com **Abstract:** The purpose of this study is to shed light on the importance of folk art as a national art, which should be with him to maintain the continuity by employing a selection of units of the Egyptian People and their meanings of symbolism in the decorative design of the T-shirt as a product commensurate with the youth of both sexes during the age (20 to 30 years), by identifying the views of all producers of clothes, textile and consumers in the proposed designs and the potential demand for purchase and implementation of a selection of them. The research samples contain 418 single distributed according to the research variables on the producers and the number (10) and intended them gentlemen producers of clothes for young people of both sexes and in particular the product T Shirts, and consumers are (408), and understood to mean members of the community of young men and young women aged (20 to 30 years) level of education between (high, medium, low), in order to know the views of samples of the research in the proposed designs and made the most important findings point to the as follows: 1 - the best designs in accordance with the views of producers in the "appropriate decoration popular designs of the proposed" order is a design (V, IX, II, XIV, XI, and IV), due to the fact that these designs bear the character of the popular in contemporary more than Other designs, and then followed in the order designs (VIII, XIII, XV, XVI, and I), and comes at the end designs (X, VII, III, and XII). 2 - the best designs in accordance with the views of producers on "the possibility of the implementation and marketing of proposed designs," the order is the design, "IV, IX, XIV, I, VI, and VI," The reason for this is that these designs can be implemented by more than a method with low costs of production "In terms of raw materials, method of implementation of the decoration, lines run inside the factories," as it gives a higher percentage of profits as a result of consumer acceptance for, and then followed in the order designs, "XII, XIII, V, II, VII", and comes in the end designs "XI, X, XV, and VIII". 3 - There are significant differences between the mean scores of the views of consumers according to the research variables "in the appropriate technical designs proposed at the level (0.01) to the (female, age from" 25 to 30 "years, higher education). 4 - There is no statistically significant difference between the averages of the views of consumers according to the research variables "sex" in the extent of consumer acceptance of the proposed designs. 5 - There are significant differences between the mean scores of the views of consumers according to the research variables "age, level of	Full Text	107

	education" in the extent of consumer acceptance of the designs proposed "at the level (0.01) for the (age of" 25 to 30 "years, higher education). **[**Rabab H. Mohammed and Sahar A. Zaghloul**. Egyptian Folk Art and its Significance as a Source of Symbolic Design Decorative Clothes Young Men and Women.** Journal of American Science 2011; 7(1):1074-1091]. (ISSN: 1545-1003). http://www.americanscience.org. **rd:** Egyptian folk art, symbolic meaning, decorative design, Clothing, young men and women		
108	**Uncertainty determination of correlated color temperature for high intensity discharge lamps** A.B. El-Bialy[1], M.M. El-Ganainy[2] and E.M. El-Moghazy[3] [1]University College for Woman for Art, science and education. Cairo , Egypt [2] National Institute for Standards (NIS), Giza, code 11211, Egypt [3] NIS and Ph.D. student in University College of Woman, Giza, code 11211, Egypt. emoghazy@yahoo.com **Abstract:** Color temperature is a description of the color of light sources. The chromaticity coordinates of the light source lying on the Planckian locus which is called (Commission Internationale de l'Eclariage, referred to as CIE) CIE diagram and the source has color temperature (in Kelvin) equal to the blackbody temperature of the Planckian radiator. For light sources that don't have chromaticity coordinates that fall exactly on the Planckian locus but lie near it. In this case the chromaticity coordinates of such sources can be representing by correlated color temperature (CCT). Uncertainty of Correlated Color Temperature (CCT) or **(T_{cp})** for high intensity discharge lamps (HID) is derived from (u, v) color coordinates. The method of the International organization for standardization (ISO) Guide is applied by Gardner to drive analytical expression for uncertainty in **u** and **v** chromaticity coordinates and an uncertainty in CCT for few Kelvins can be achieved. The color temperature standard achieved with the uncertainty is. ± 11.48 K for mercury lamp, ± 3.44 K for sodium lamp and ± 6.4 K for metal halide lamp). [A.B. El-Bialy, M.M. El-Ganainy and E.M. El-Moghazy, Uncertainty determination of correlated color temperature for high intensity discharge lamps. Journal of American Science 2011; 7(1):1092-1096]. (ISSN: 1545-1003). http://www.americanscience.org. **Key words**: lamp, correlated color temperature, Uncertainty and luminous flux	Full Text	108
109	**Genotoxic Effects of Acrylamide in Adult Male Albino Rats Liver** **Khlood M. El- Bohi[1], Gihan G. Moustafa[1], Nabela I. El sharkawi[1] and *[2]Laila M. E. Sabik** [1]Dept of Forensic Medicine &Toxicology. Faculty of Veterinary Medicine , Zagazig University, Egypt. *[2]Dept. of Forensic Medicine & Clinical Toxicology. Faculty of Medicine, Zagazig University, Egypt. *Lailasabik714@hotmail.com **Abstract:** Background: Acrylamide is a common chemical which is used in both industrial and laboratory processes. It is formed in heated starchy foods especially potato products. Aim of the work: The aim of the present study was to clarify the possible involvement of genotoxic mechanisms in acrylamide-induced hepatotoxicity by measuring the role of cytochrome P450 2E1 (CYP2E1) gene protein and mRNA in rats intoxicated with acrylamide and recording the DNA changes in their hepatic tissues by the *in vivo* alkaline single cell gel electrophoresis (Comet assay). Material and Methods: Thirty mature male albino rats were used in this study. Rats were classified randomly into three groups; the first group daily received 50 mg/kg acrylamide orally for 21 days. The second group received twice the previous dose (100 mg/kg) by the same route and duration and the third group was administered distilled water and kept as control. Results: The results revealed that, acrylamide caused marked alterations in animal behaviour and mortality % in both treated groups which reached 30% (in the first group) and 40% (in the second group) . Acrylamide elicited a highly significant increase in serum AST and ALT, while a significant decrease of total protein, albumin and globulin levels were recorded. Acrylamide caused down regulation of both CYP 2E1 protein and its mRNA expression concomitant with a dose dependent significant increase in number of DNA single strand breaks. Histopathological investigation revealed necrotic and degenerative changes in the liver of acrylamide treated rats. Recommendation: Acrylamide exposure either occupationally or dietary must be restricted. In addition to, raising awareness of people about its hazards. **[**Khlood M. El- Bohi, Gihan G. Moustafa, Nabela I. El sharkawi and Laila M. E. Sabik. **Genotoxic**	Full Text	109

	Effects of Acrylamide in Adult Male Albino Rats Liver. Journal of American Science 2011; 7(1):1097-1108]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Acrylamide, Glycidamide, Genotoxicity, CYP2E1, Comet assay		
110	**Detection of Community Acquired Methicillin Resistance Staphylococcus aureus among Staphylococcus aureus isolates.** Ola Kader [1], Samia Ebid [2], Nancy Mostafa [21] Shimaa El Sayed [2] and Abeer Ghazal [1] [1] Microbiology Department and [2]Applied Medical Chemistry Department, Medical Research Institute, Alexandria University. **ABSTRACT:** The rates of MRSA infections in the hospital, as well as the disease in the community, have continued to rise. Staphylococcal cassette chromosome *mec (*SCC*mec)* is a variable genetic element that contains the methicillin resistance determinant, *mecA*. SCC*mec* typing is one of the most important molecular tools available for distinction between community-acquired MRSA and HA-MRSA occurring on a worldwide basis. CA-MRSA has been reported to carry the loci for Panton Valentin leukocidin (PVL) in high frequency in association with the type IV *SCCmec*. **Aim of this study was to** differentiate between HA-MRSA and CA-MRSA by detection of SCC*mec* and determination the prevalence of PVL gene among MRSA isolates. **Material &methods**: A total of 34 *Staphylococcus aureus* isolates were included in this study. Susceptibility of Staphylococci was determined by, Disc diffusion method including methicillin, oxacillin and cefoxitin discs. Penicillin Binding Protein (PBP_{2a}) Latex Agglutination test was done to detect the presence of PBP_{2a} responsible for methicillin resistance. In addition genotypic identification of MRSA was carried out by detecting *mec* gene by real time PCR. Conventional PCR was carried using different set of primers for the amplification of SCC *mec* for differentiating the HA-MRSA and CA-MRSA; moreover detection of *PVL* as virulence factor was also done. **Results**: The antibiotic sensitivity of CA-MRSA ranged from (11.76% for ceftazidime) to (47.06% for Imipenem, Erythromycin and Gentamycin); while the sensitivity of HA-MRSA ranged from (2.94% for Amoxicillin and Ampicillin/sulbactam) to (29.41% for Amikin). Out of 34 *S. aureus* strains; 26(76.47%) isolates were found to be resistant to oxacillin disc, 30(88.24%) isolates were resistant to methicillin; and all strains were resistant to cefoxcitin disc. All MRSA strains were confirmed to be methicillin resistant by detection of *mecA* gene using real time PCR. Out of 34 MRSA strains 32 (94.12%) were PBP_{2a} producer. In the present study, though, the majority (25out of 34) of our strains were not SCC mec typable, yet among the nine typable strains the six hospital strains belonged to type II and III as reported in the literature and the three CA-MRSA belonged to the novel type V reported by other workers to be associated with CA-MRSA and the only PVL positive CA MRSA strain was untypable. **[**Ola Kader, Samia Ebid, Nancy Mostafa, Shimaa El Sayed and Abeer Ghazal. **Detection of Community Acquired Methicillin Resistance Staphylococcus aureus among Staphylococcus aureus isolates.** Journal of American Science 2011; 7(1):1109-1117]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords:** Community; Methicillin; Resistance; Staphylococcus; Staphylococcus; aureus	Full Text	110
111	**Comparative Antioxidant Activity Study of Some Edible Plants Used Spices in Egypt.** Hala, M. Abdou Biochemistry Department, National Research Center, Dokki, Cairo, Egypt, E-mail: abdou.hala@yahoo.com **ABSTRACT:** There is an increasing demand for natural antioxidants to replace synthetic additives in the food industry. Many spices have been shown to impart an antioxidative effect in foods. The spices are defined as dry plant material that is normally added to food to impart flavor. Methanol, methanol and water (1:1), water (37°C), water (100°C) extracts of ten edible plants (spices: cumin, chili, pepper, nutmeg, garlic, cloves, ginger, coriander, onion and thyme) were tested as extractants of total polyphenols, antioxidant activities. Antioxidant activities of the extracts were evaluated by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay and a β-carotene bleaching assay. Methanol extract of cloves showed the highest total phenolics content (171.8 mg garlic acid equivalents/100 g dry weight cloves powder). Total antioxidant activity of the ten spices determined by radical scavenging (DPPH) were ranged from (26.19-85.31%). The antioxidant activity by β-carotene-lenoleic acid were ranged from (36.55-85.43%). Methanol	Full Text	111

	extract of cloves showed the highest antioxidant activity by DPPH of β-carotene-linoleic acid methods were (85.31, 85.43% respectively). **[Hala, M. Abdou. Comparative Antioxidant Activity Study of Some Edible Plants Used Spices in Egypt.** Journal of American Science 2011; 7(1):1118-1122]. (ISSN: 1545-1003). http://www.americanscience.org. **Key Words**: cumin, chili, papper, nutmeg, garlic, cloves, ginger, coriander, onion, thyme, total phenolics, antioxidant activity, solvent.		
112	**One Country, Two Systems: The Dualistic Land Tenure System in Sierra Leone, and the Need for Reform** Victor Tamba Simbay Kabba[1, 2] and Jiangfeng Li[3] [1]Department of Land Resources Management, Faculty of Economy and Management, China University of Geosciences, Wuhan, 430074, Hubei Province, China Victor_kabba@yahoo.co.uk 0086-15827480592 [2]Institute of Geography and Development Studies, School of Environmental Sciences, Njala University, Republic of Sierra Leone [3]Department of Land Resources Management, Faculty of Earth Resources, China University of Geosciences, Wuhan, Hubei 430074, China **Abstract:** Several studies have indicated a strong link between poverty and insecure land tenure. In Sierra Leone like other former British colonies, two separate land tenure systems exist: an imposed British tenure in the western area, and a customary system in the rest of the country. Whilst the former allows freehold tenure, the latter does not. Seventy-five percent of its population are rural, and invariably depends on agriculture for livelihood sustainability. Statistics also show that women who form the bulk of this population are involved in food production. One of the reasons identified why the country is unable to feed its population is the existence of the customary system. In this work, we discussed the two land tenure systems in the country, and analyzed the shortcomings of the customary tenure in detail. Data were mainly desktop literature. We looked at similar cases elsewhere and drew our conclusions. . We discovered that the customary system is not only discriminating against women, and other citizens (from other parts of the country), but discourages investment in agriculture and other land uses in rural areas. It is therefore a threat to food security and rural development in general. It also provokes tension between citizens from the western area, and those from the rest of the country. If the Poverty Reduction Strategy Paper, VISION 2025 and the Millennium Development Goals are to be realized, it is important that authorities step up and reform this customary system, and encourage more access to land, say freehold tenure. **[**Victor Tamba Simbay Kabba and Jiangfeng Li**. One Country, Two Systems: The Dualistic Land Tenure System in Sierra Leone, and the Need for Reform.** Journal of American Science 2011; 7(1):1123-1129]. (ISSN: 1545-1003). http://www.americanscience.org. **Keywords*:*** land tenure, tenure insecurity, freehold, customary tenure, women, poverty, discrimination, rural areas	Full Text	112

The Effects of Processing on the Anti-Nutritional Properties of 'Oze' *(Bosqueia angolensis)* Seeds

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Abstract: 'Oze' (*Bosqueia angolensis*) is found in the tropical rain forest and grows in thick humid forest of undisturbed land. It belongs to the family *Moracea.* Wholesome 'oze' (*Bosqueia angolensis*) seeds were given different treatments, which included blanching, cooking, roasting and malting. The samples obtained from these treatments were analyzed for anti-nutritional properties. The 'oze' seeds had up to eleven anti-nutrients with alkaloids (2730 mg/100 g) and Total phenols (2500 mg/100 g) predominating. Except for Total phenols and Trypsin inhibitors (37.3 TIU/100 g) all the other anti-nutrients were found more in the hulls than the edible cotyledons. Also all anti-nutrients except phytates and oxalates were eliminated by malting. [Journal of American Science. 2011;7(1):1-6]. (ISSN: 1545-1003).

Key words: anti-nutritional factors, malting, blanching,

1. Introduction

Oze (*Bosqueia angolensis*) referred to, as the "hospitality tree" in the cultural Igbo Community is a member of the botanical family, *Moracea*. It is a tropical rain forest tree and grows in the thick, humid forest of undisturbed land (Keay, 1989). The tree grows up to 30 – 40 meters high as it competes with other hard wood for sunlight. Its green glossy leaves resemble those of 'Ogbono' (*Irvingia gabonensis*); but it is readily distinguished by the remarkably abundant latex flow observed immediately at a slash of its node. This plant called "Oze" in the Igbo speaking states of South Eastern zone of Nigeria is called "koko eran" in the Yoruba speaking states of South Western states of Nigeria.

In most developing tropical countries the food situation is worsening owing to increasing population; shortage of fertile land, high prices of available staples and restrictions on the importation of food (Sadik, 1991; Weaver, 1994). This has resulted in a high incidence of hunger and malnutrition, a situation in which children and women, especially pregnant and lactating women, are most vulnerable (Coulter *et al.*, 1988; Pelletier, 1994). Predictions of future rates of population increase and food production emphasize the seriousness of this problem (FAO, 1990). There seems to be no immediate single solution to the problem of food sufficiency, thus interdisciplinary approach is necessary (Avery, 1991). All information on new sources of food will be of value in dealing with the food problem.

While every measure is being taken to boost food production by conventional agriculture, a lot of interest is currently being focused on the possibilities of exploiting the vast numbers of less familiar food plant resources existing in the wild (RAO, 1994). Many such plants have been identified, but the lack of data on their chemical composition has limited the prospects for their broad utilization (Vijayakumari *et al.*, 1994; Viano *et al*., 1995). Most reports on some lesser-known and unconventional crops indicate that they could be good sources of nutrients and many have the potentials of broadening the present narrow food base for human (Janick and Simon, 1990).

The aroma of roasted Oze seed is reminiscence (resembles) that of its family member, African breadfruit; but its usual traditional dehulling process is more laborious (drudgery) than that of African breadfruit seeds. This factor has limited the traditional processing of 'Oze' to mere hot-ash roasting and a limited consequent utilization as snacking kernels, just as roasted cashew nuts. Thus 'Oze' though aromatically and morphologically more like African breadfruit is utilized mainly as indigenous snacking nuts just like cashew nuts.

Usually, the consumption of hot ash roasted Oze seed results in high gasing phenomenon, suggesting the presence of some anti-nutritional factors. Also the gas has a smell reminiscence of hydrogen sulphide suggesting the presence of sulfur containing amino acids which are among the essential amino acids needed in our daily diet.

Application of different processing methods to 'Oze' seed will give some information, which may increase the utilization of Oze seeds and enhance its potential in food formulations. It is envisaged that a more preferred process for the elimination or reduction of any detected anti-nutritional factor may be found for the production of safer 'Oze' product.

The objective of this study therefore is to investigate the effects of given treatments on the anti-nutritional compounds identified in 'oze' seeds.

2. Materials and Methods

2.1 Materials Collection and Preparation

The 'oze' seeds with intact pulp were obtained from abandoned shrine spots at Ubomiri in Mbaitoli Local Government Area and Umuchima in Ideato South L.G.A both in Imo State. The pulp was washed off with water by rubbing with the hands. The seeds were then dried in the oven at 50°C – 55°C for 24 hours. The cleaned dry seeds were then given different treatments, which included blanching, cooking, roasting and malting after which they were dehulled. Blanching was carried out for 4,6, and 8, min respectively; while cooking was carried out at boiling temperatures for 20, 40, and 60 min respectively. Roasting was carried out in the oven at a temperature of 150°C for 45 minutes. Malting was done by steeping the 'oze' seeds for 24 hours in water at a ratio of 1:2; (seed to water) then germinating the seeds at room temperature for 3 weeks before drying. All samples were dehulled and then milled using the manual grinder (Corona model), sieved to obtain fine powder, which were packaged in airtight plastic containers until needed for analysis.

3. Determination of Anti-Nutritional Factors in 'Oze' Seed Flour

Anti-nutritional factors were determined in the 'oze' seed flour as follows:

3.1 Tannins

Ten grams of each sample was weighed into a 100ml conical flask and 50ml of methanol was added. The flask was stoppered, shaken and left for 24 hours. The contents of the flask were shaken after extraction and the solid particles were allowed to settle. After filtration, the volume of the extract was measured. To 1ml portion of the extract, 5ml of fresh vanillin – HCl was added and the solution was left to develop colour in 20 minutes. The absorbance was measured at 500nm against a reagent blank using corning 253 spectro-photometer.

3.2 Phytate

The phytic acid in the samples was precipitated with excess $FeCl_3$ after extraction of 10g of each sample with 100ml 0.5N HCl. The precipitate was converted to sodium phytate using 2ml of 2% NaOH before digestion with an acid mixture containing equal portions (1ml) of conc. H_2SO_4 and 65% $HCl0_4$. The liberated phosphorus was measured colorimetrically at 620nm after colour development with molybdate solution.

3.3 Hydrogen cyanide determination (AOAC, 1984)

Two grams of the sample was weighed into a flask and 100ml of distilled water added to it and allowed to hydrolyse for 1hr. 10ml of 2.5% NaOH was measured and carefully poured into the sample holder. The soxhlet apparatus was set up and was distilled into the sample holder containing the 2.5% NaOH until about 70ml was collected. It was carefully transferred to a 100ml volumetric flask and the sample holder rinsed with distilled water successively and also poured into volumetric flask. It was made up to the mark. Twenty-five milliliters (25ml) of the distillate was pipetted into a conical flask, 2ml of 6M NH_4OH was added and 0.5ml of 10% KI soln, it was titrated with 0.02M$AgN0_3$ to a first turbid colour

NB: 1ml of 0.02 M $AgN0_3$ = 1.08mg cyanide.

3.4 Trypsin inhibitor

This was done using the spectrophotometric method, described by Amtfield *et al*., (1985).

A measured weight (10g) of the test sample was dispersed in 50ml of 0.5 M Nacl solution and stirred for 30min at room temperature. It was centrifuged and the supernatant filtered through Whatman No 42 filter paper. The filtrate was used for the assay.

Standard trypsin was prepared and used to treat the substrate solution (N-α - benzoyl - Dl – arginine – p – anilide; BAPA). The extent of inhibition was used as a standard for measuring the trypsin inhibitory activity of the test sample extract. Into a test tube containing 2ml of extract and 10ml of the substrate (BAPA) 2ml of the standard trypsin solution was added. Also 2ml of the standard trypsin solution was added in another test tube containing only 10ml of the substrate. The latter served as the blank.

The content of the tubes were allowed to stand for 30min and then the absorbances of the solution measured spectrophotometrically at 410nm wavelength. One trypsin activity unit inhibited is given by an increase of 0.01 absorbance unit at 410nm.

$$\text{Trypsin unit inhibited} = \frac{Au}{As} \times 0.01 \times F$$

Where Au = Absorbance of test sample

As = Absorbance of standard (uninhibited) sample

F = Experimental factor given as

$$\frac{Vf}{Va} \times \frac{1}{W}$$

Vf = Total volume of extract

Va = Volume of extract analysed

W = Weight of sample analysed

3.5 Alkaloids (AOAC, 1984)

Five grams of the sample was dispersed in 10% acetic acid solution in ethanol to form a 1:10 w/v dispersion. The sample was stirred for every 30min for 4hr. The mixture was filtered using Whatman filter

paper. The filtrate was concentrated by evaporation over a water bath until it remained ¼ of the original volume.

Concentrated ammonia solution was added in drops and the alkaloid in the filtrate was precipitated. It was filtered using a pre-weighed filter paper (Whatman). The filter paper and the precipitate were dried in the oven at the temperature of 60^{o}C, cooled in a desicator and reweighed. The difference between the mass of filter paper plus the precipitate and the mass of filter paper alone gave the mass of the alkaloid.

$$\% \text{ Alkaloid} = \frac{\text{Mass of the alkaloid}}{\text{Mass of sample}} \times \frac{100}{1}$$

3.6 Determination of Saponins (AOAC, 1984)

One gram metric method employing the use of soxhlet extractor and two different organic solvents was easily used. The first solvent extracted lipids and interfering pigments while the second solvent extracted saponins proper.

Five grams of the ground sample was weighed into a thimble and transferred into the soxhlet extractor chamber fitted with a condenser and flask. Some quantity of petroleum spirit (boiling point 40 – 60^{o}C) enough to cause a reflux was put into the flask. Extraction continued for 3hr, which extracted the lipids and interfering pigments. The defatted material in the thimble was then used for the second extraction for saponins.

A fresh preweighed flask was fitted into the soxhlet apparatus (bearing the thimble containing the defatted sample) and methanol was put in the flask. The quantity of methanol should be enough to reflux and flush for 3 hours. The saponin was exhaustively extracted by heating the flask on a heating mantle. After the thimble and its content was removed and the methanol recovered leaving the saponin and little quantity of methanol in the flask. It was then taken to an oven and kept at slanting position at a temperature of 70^{o}C to evaporate the residual methanol. The flask and content was weighed and the difference between the flask plus saponin and flask alone was the mass of saponin extracted.

$$\text{Calculation \% Saponin} = \frac{\text{Mass of saponin in g}}{\text{Mass of sample}} \times \frac{100}{1}$$

3.7 Total Steroids

The total steroids was determined colorimetrically with reference to the saponin content. The saponic crystals were dissolved in a 50 ml formaldehyde- conc. H_2SO_4 mixture and the absorption was measured at 470nm. The steroid content was calculated as follows:

$$\% \text{ Steroids} = \frac{\text{Abs.}}{\text{wt of sample}} \times \frac{100}{1}$$

3.8 Oxalate determination

Five grams of the sample was weighed into a 100ml beaker, 20ml of 0.30N HCl was added and warmed to (40 – 50^{o}C) using magnetic hot plate and stirred for one hour. It was extracted three times with 20ml of 0.30N Hcl and filtered into a 100ml volumetric flask. The combined extract was diluted to 100ml mark of the volumetric flask.

The oxalate was estimated by pipetting 5ml of the extract into a conical flask and made alkaline with 1.0ml of 5N ammonium hydroxide. A little indicator paper was placed in the conical flask to enable us know the alkaline regions. It was also made acid to phenolphtalein (2 or 3 drops of this indicator added, excess acid decolourizes solution) by dropwise addition of glacial acetic acid. 1.0ml of 5% $CaCl_2$ was then added and the mixture allowed to stand for 3hrs after which it was then centrifuged at 3000rpm for 15min. The supernatants were discarded and the precipitates washed 3 times with hot water with thorough mixing and centrifuging each time. Two milliliters of 3N H_2S04 was added to each tube and the precipitate dissolved by warming in a water bath (70 – 80^{o}C). The content of all the tubes was carefully poured into a clean conical flask and tiltrated with freshly prepared 0.01N KMnO4 at room temperature until the first pink color appeared throughout the solution. It was allowed to stand until the solution became colourless. The solution was then warned to 70 – 80^{o}C and titrated until a permanent pink colour that persisted for at least 30 seconds was attained.

3.9 Total Phenols

Two grams of sample was weighed into a separator containing 30ml of chloroform plus 10ml of water and 1ml of diluted sulphuric acid in the ratio 1:1 (acid to water) and was vigorously shaken. The chloroform layer was drained off into 100ml volumetric flask. The extraction was repeated thrice with 20ml portions of chloroform. The three extracts were combined and made up to 100ml with chloroform and mixed. The chloroform extract solution (20ml) containing about 400mg diethyl-stilbestrol was transferred to small Erlenmeyer flask and evaporated to dryness on steam bath. The extract was cooled in a vacuum dessicator for about 10min and 20ml of alcohol was added, and residue dissolved by swirling. After 15min, the solution was mixed with 10 drops of diluted sulphuric acid and potassium at a ratio of (1:1) and the mixture cooled. Five drops of 10% sodium nitrate ($NaNO_3$) solution was added and the preparation was allowed to stand for 45mins with occasionally mixing. Some quantity was washed into 25ml volumetric flask with about 20ml alcoholic ammonium hydroxide (NH_40H) solution (equal volume of alcohol + diluted NH_40H) (4+6). Next the sample was cooled in ice bath

and allowed to stand at room temp for 1hr. Then was diluted to volume with dilute NH_40H solution and mixed. A white precipitate formed, was filtered through a dry filter paper. The clear yellow alkaline solution obtained was analysis at 420nm wavelength using spectronic 200 spectrophotometer against alcohol (1:2) as blank.

The percentage total phenols was calculated as follows:

$$\% \text{ Total Phenol} = \frac{\text{Sample Abs.}}{\text{Sample wt}} \times \frac{100}{1}$$

3.10 Oligosaccharides (Starchyose and Raffinose)

The method of Balagopalan *et al.*, (1988) was used. One gram of sample was boiled in 10mls of M HCl solution until it was negative to iodine starch test. It was centrifuged and the hydrolysate (Supernatant) used for the analysis. 2mls of the hydrolysate was mixed with 4mls of anthrone reagent in a test tube and boiled for 10min in a water bath while covering the test tubes. After boiling, the mixture was filtered and diluted with distilled water. Similarly, a standard sugar solution (glucose) was prepared and treated as described above and the absorbances of both the samples and sugar solutions were read spectrophotometrically at 625nm using Genway spectrophotometer against a blank reagent at zero.

The Oligosaccharides content was calculated as:

$$\text{Percent sugar} = Au/As \times C \times F$$

Au = Absorbance of test sample
As = Absorbance of Std sugar solution
C = Conc. of sugar solution
F = Experimental factor given by

$$\frac{Vf}{Va} \times D \times \frac{100}{w}$$

Vf = Total filtrate volume
Va = Volume of aliquot analysed
D = Dilution factor
W = wt of sample used

4. Results and Discussions

4.1 The Anti-nutritional Components in "Oze" Seed Flour Samples

Eleven anti-nutritional components at different levels were found in raw 'oze' seed flour. There were trypsin inhibitors, tannins, phytates, oxalates, saponins, hydrogen cyanide, alkaloids, stachyose, raffinose, phenols and steroids (Table 1). With regards to levels observed, the most prominent among them were the alkaloids, (2730mg/100g), phenols (2500mg/100g), saponins (840mg/100g) and hydrogen cyanide (280mg/100g), while the oxalates (2.80mg/100g) and tannins (3.11mg/100g) were the least.

Interestingly, in all except phenols, the anti-nutritional factors identified were more in the raw hulls than in the raw edible cotyledons, and even there, the alkaloids (7640mg/100g), saponins (1920mg/100g), hydrogen cyanide (400mg/100g), phenols (400mg/100g) and steroids (300mg/100g) were more prominent. Heat treatment and malting affected all the nutritional stress factors and all decreased with increased period of heating, though not at the same rate of reduction. For instance, at 6min blanching period, a reduction of 92.86% (840mg/100g – 60mg/100g), was achieved in saponins, and reduction of 94.87% (2730 – 140mg/100g), 99.6% (2500 – 10mg/100g), 63.71% (15.68 – 5.69mg/100g) and 46.62% (3.11 – 1.66mg/100g) were achieved in alkaloids, phenols, phytates and tannins respectively, while complete elimination was achieved in hydrogen cyanide, oxalates, stachyose and raffinose. These results were in agreement for other similar seed as reported by Uzogara *et al*., (1990); Jood, *et al*., (1989) and Osagie, (1998). With the exception of tannins and phytates which had 27% and 22% respectively of their raw material values retained at 20min cooking period, all other factors were completely eliminated or degraded at 20min cooking period. All factors were destroyed after 40min cooking. Roasting (dry heat treatment) for 45min destroyed all factors with the exception of tannins and phytates. Specifically, samples roasted for 45mins had 19.94% and 17.35% of their raw material of tannins and phytates (respectively) retained. Also the malting operation destroyed all factors with the exception of phytates and oxalates. Malted samples retained 2.55% of its original level of phytates and 72.14% of its original level of oxalates. These findings indicated that generally moist heat application was more effective in eliminating the anti-nutritional factors in oze seeds than dry-heat application. It was interesting to observe that with exception of phenols where the raw cotyledon level (2500mg/100g) was 6.3 times higher than the level (400mg/100g) found in the hulls, but for all other anti-nutritional components studied, the hulls had levels ranging from 1.4 – 7.9 times higher than those observed in the raw cotyledons. For instance, the tannin level (20.12mg/100g) in hulls is 6.5 times of its level (3.11mg/100g) in cotyledon and hydrogen cyanide level (400mg/100g) in hulls is 1.4 times of its level (280mg/100g) in the cotyledons. There were significant differences ($P<0.05$) between the levels of each factor in the hulls and the raw cotyledons. There were also significant differences ($P<0.05$) between the levels of each factor in the raw sample and samples given moist heat for up to 6min.

The raw cotyledon had 37.3 TI units/100g sample and like other anti-nutritional components, this decreased as heat treatment progressed. Specifically, a reduction of 60.1% (from 37.3 – 0.74 TI units/100g) was achieved at 6min blanching period, reaching total trypsin inhibitor elimination after 8min of heat treatment. As observed for total phenols, the hulls had

lower level (9.70 TI units/100g) of trypsin inhibitor than the raw edible cotyledons. Malting, roasting (45min) and cooking (≥ 8min), completely eliminated trypsin inhibitory effect.

Tannin affect the digestive tract and their metabolites are toxic (Ene-obong, 1992). The precise toxic amount of tannin to cause depression in humans is not known yet. The values obtained in 'oze' seed flour were quite lower than the values of Ezeh (1992) for legumes.

For hydrogen cyanide the fatal dose in food is 50mg/100g which is higher than what was obtained in 'oze' seed cotyledons (2.8mg/100g). Such illnesses arising from its excesses like gasping, staggering, paralysis convulsion could be avoided.

Phytate, a chelator of cations and found in all seeds causes reduction in protein availability (Macrae and Joslyn 1993) but was reduced by germination (Oksana and Bills 1984).

As saponins haemolyse red blood cells, its elimination by heat makes it safe for human consumption. Alkaloids which causes depressed growth was eliminated through heating.

Hydrolysis of oligosaccharides with formation of simple diasaccharides and monosaccharides or other compounds, decreases its levels. Dehulling also helped to decrease them thereby reducing flatulence.

Table 1. Mean values of the anti-nutritional components in 'Oze' seed flour sample

Samples	Trypsin Inhibitors T.IU/100g	Tannins mg/100g	Phytates mg/100g	Oxalates mg/100g	Saponins mg/100g	HCN mg/100g	Alkaloids mg/100g	Stachyose mg/100g	Raffinose mg/100g	Total Phemols mg/100g	Total steroids mg/100g
Raw	37.3^{e}	3.11^{d}	15.68^{d}	2.80^{b}	840^{b}	2.80^{b}	2.730^{cd}	38^{ab}	15^{ab}	2500^{c}	38.0^{c}
4 min blanch	14.90^{d}	2.28^{c}	6.66^{c}	0.20^{a}	100^{b}	0.00^{a}	270^{c}	3^{a}	2^{a}	50^{b}	8.0^{b}
6 min blanch	0.74^{b}	1.66^{c}	5.69^{c}	0.00^{a}	60^{b}	0.00^{a}	140^{b}	0.00^{a}	0.00^{a}	10.0^{a}	4.0^{ab}
8 min blanch	0.00^{a}	2.24^{b}	4.62^{c}	0.00^{a}	20^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
20 min cook	0.00^{a}	0.83^{b}	3.39^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
40 min cook	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
60 min cook	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Roasted (45 min)	0.00^{a}	0.62^{b}	2.72^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Malted	0.00^{a}	0.00^{a}	0.40^{a}	2.02^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Hulls	9.70^{c}	20.12	35.98^{e}	7.80^{c}	19.20^{d}	400^{c}	7640^{e}	58.0^{ab}	52.0^{a}	400^{d}	300^{c}
LSD	12.21	5.189	9.52	2.14	0.53	0.12	2.08	0.18	0.14	6.65	0.08

Note: Means with different superscripts along the column have significant difference at $P<0.05$.

Conclusion

The results obtained from the project have shown that *Bosqueia angolensis* popularly known as 'oze' in Igbo speaking community yields flour which contains some naturally occurring toxins /antinutrients, the level at which they occur coupled with the natural detoxification methods and loss of these toxins during processing make their presence to be of little concern.

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Screening of Leguminous Plants for VAM Association and Their Role in Restoration of Degraded Lands

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Abstract: In present study, 50 leguminous plant species were assessed for association of Vesicular-Arbuscular Mycorrhizal fungi. For this, fine roots of these plants were carefully dug out, washed and stained using root clearing methods and observed under microscope. Out of 50 species screened, 5 showed no VAM association, 2 species showed very low level of colonization (> 20%), 17 species showed 20 to 49 % colonization, 24 species showed 50 to 69 % colonization and only 2 species showed very high level of colonization i.e. <70%. Most of the plant showed hyphae with vesicle/arbuscles. However in five species viz. *Bahunia retusa, Crotolaria albida, Desmodium elegans, D. heterocarpon* and *Vicia rigidula* only hyphae of mycorrhizal fungi is present. Thus, the legumes with high to very level of VAM colonization can be use in restoration of degraded lands. [Journal of American Science. 2011;7(1):7-11]. (ISSN: 1545-1003).

Keywords: Legumes, roots, vesicles, arbuscles, colonization

1. Introduction

Restoration is defined as a tactic employed to return degraded lands to its original condition. Of the total earth's surface 78% of the land area is unsuitable for agriculture. Out of the remaining land, about 9% suffers from physical, chemical and biological constraints requiring special management practices. Since, land degradation process involve loss of vegetation and accelerated run off and soil loss, they require assistance for their restoration and regeneration. Mycorrhizal fungi and nitrogen-fixing bacteria are among the major beneficial components of soil microbial community, which contribute to plant growth and survival by reducing stresses through symbiosis (Sylvia and Williams 1992). These plants have special nutritional relationship between the two symbionts: the high phosphorus requirement of the nitrogen fixing root nodule and the high nitrogen requirement of the chitin walled VAM fungi and the high carbon requirement of both. Since each symbiont can supply the other's need in excess, the endophytes can bring about when the association is grown in nutrient-deficient soil (Norris et al 1994). In addition, VAM mycelia can extend to a long distance and can link the rhizosphere and mycorrhizosphere of different plant species; it can make common pool of the available nutrients of the other plant species (Norris et al 1994). This way, the nutrients released into the overlapping mycorrhizosphere by plant root exudation or by root and nodule decay become available for non-N_2 fixing plants (Simard et al 1997), which can play important role and facilitates the survival and growth of other plant species. Such observations led to a view of the legume microsymbionts as biological substitutes for fertilizers.

The present study assessed VAM colonization in leguminous plants of Kumaun Himalayan region. This study has greater implications as results of present study can be used to stabilize many degraded areas in the region, which is a frequent phenomenon all over the Himalaya.

2. Material and Methods

This study was conducted in Kumaun Himalayan region of Central Himalaya, India. Kumaun is situated between the latitude of $28^{o}44'$ - $33^{o}49'$ N and longitudes of 78^{o} 45' - $81^{o}05'$ E covering an area of about 21,033 km^2 ranging from 300 m to the 5400 m elevation. Broadly this region can be differentiated into four physiographic domins viz. the outer Himalaya with Terai and Bhabhar belts and Siwalik ranges; the Lesser Himalaya; the Great Himalaya and the Trans Himalaya (Jalal 1988). The climate of the region is governed by the monsoon and the year can be divisible into summer (April- mid June), rainy (mid-June- September) and winter (October - Feburary). The annual rainfall is about 2500 mm in most of the places, of which about three-fourth occur during rainy season (Singh and Singh 1992).

3. Material and Methods

The field surveys were conducted in different forest sites and leguminous plants growing in the region were identified. For each species fine terminal roots

were collected from different places of root system selecting five plants at random and collected in separate polythene and brought to the laboratory. Roots were gently washed, cut into one cm segments and preserved in formalin-acetone alcohol (FAA) in the ratio of 90:5(v: v: v). These preserved root samples were used for studying the level of VAM colonization. Root clearing method (Phillips and Haymann 1970) was used to stain the roots by heating in KOH. The root sample stored in FAA were washed thoroughly with tape water and placed in test tubes and 10% KOH solution was added. These test tubes were heated at 90^{o} C for one hour in a water bath. After heating, the KOH solution was poured off from the test tube and roots were rinsed with several changes of distilled water. Then roots were placed in 1% HCL solution for 5 minute to acidify the roots. The roots were again rinsed several times. After rinsing, the roots were stained with 0.1% trypan blue staining solution and heated again for 5 minutes. Extra stain was poured off and lactophynol solution was added. Heavily pigmented roots were bleached prior to staining with alkaline H_2O_2 (Phillips and Haymann 1970). The root segment were then pressed gently and observed under a microscope for VAM colonization. Percent VAM colonization was calculated using Nicolson 'simple formula (1955; see; Gupta and Mukerji 1999):

$$\text{Colonization (\%)} = \frac{\text{No. of root segments colonized with VAM}}{\text{Total number of root segments observed}} \times 100$$

The colonization was categorized in the following groups based on the intensity of infection:

1. **Excellent:** Mycelia/vesicle/arbuscules present on whole surface of the root bits (1 cm length) in very large number.
2. **Good:** Mycelia/vesicle/arbuscules present on whole surface of the root bits.
3. **Moderate:** Mycelia/vesicle/arbuscules present sparsely on the surface of the root bits.
4. **Poor:** Mycelia/vesicle/arbuscules present on root surface only in few numbers.
5. **Nil:** Mycelia/vesicle/arbuscules totally abscent.

3. Results

A total of 50 plant species belonging to 3 families of legumes (Papilionaceae, Caesalpiniaceae and Mimosaceae) were surveyed for VAM association. Out of 50 plants sampled, 5 plants namely *Crotolaria madicaginea, Lathyrus aphaca, Vicia pallida, V. tetrasperma* and *Pterocarpus marsupium* did not possess any VAM association. Two plant species viz. *Crotolaria albida* and *Desmodium heteropogon* possessed very low level of association (less than 20%). The sparse infection in these species could be attributed to the continuous cover of root hairs. Baylis (1975) suggested that plants with greater number of root hairs would have less dependence on mycorrhiza. Intermediate level of association (20-49%) was recorded in 17 plant species. Some of the important species in this range were *Astragalus leucocephalus, Lespedeza gerardiana, Bahunia variegata* etc. High level of VAM colonization (50-69%) was recorded in 24 plants including *Indigofera dousa, Cassia floribunda, Albezia lebbeck* etc. Two plants *Indigofera heterantha* and *Trifolium repens* possessed very high (> 70%) level of colonization (Table 1). There were differences between the different species of same genus in the percentage of root infected. For example, in *Indigofera dousa* the infection was 64% while in *I.heterantha* the infection was 72% (Table 1).

Five plants showed presence of only VAM hyphae in their roots where neither arbuscules nor vesicles were recorded. They were *Bahunia retusa, Crotolaria albida, Desmodium elegans, D. heterocarpon* and *Vicia rigidula.* Rest of the species contained hyphae along with vesicles and or arbuscles (Table 1). Although many species (25%) of the legume in the present study possessed arbuscules, the number of plants possessing vesicles was higher than plants bearing arbuscules. These results suggested that roots of majority of the plants colonized were mature as vesicles are storage organs and generally produced in the older region of the infection.

Table 1.Vesicular Arbuscular Mycorrhizal colonization in some leguminous plants of Kumaun Himalaya.

Species	Habit	Distribution	Habitat	Percent of root segment colonized			Intensity of colonization*
				A	B	C	
Family Papilionaceae							
Astragalus chlorostachys Lindl	Erect Shrub	2000-3600	As an undergrowth of birch-rhododendron forest	48	55	50	3
A. leucocephalus Garh.ex Benth	Herb	1600-2500	Shady and dry places	24	45	32	3
Argyrolobium flaccidum (Royle) Joub.	Prostrate herb	Upto 2800	On the edges of miscellaneous forest and open grassy localities	66	70	55	1
A. roseum (Camb.) Joub.	Prostrate herb	Upto 2300	Common on open grassy localities	52	55	32	3
Cajanus mollis (Benth.)Van der Maessen	Herb	Upto 2000	Shady dry and sunny places	56	62	55	3
Crotolaria albida Heyne.ex Reth	Herb	Upto 2000	Open grassy slopes	10	15	-	4
C. madicaginea Lamk.	Erect herb	Upto 1300	Open grassy places	-	-	-	5
Dalbergia sissoo Roxb	Tree	Upto 1500	Often planted roadsides	25	36	16	3
Desmodium elegans DC	Shrub	1500-2000	In oak forests and scrub jungles	0	46	0	4
D. floribundum D. Don	Erect undershrub	Upto 2600	Common in oak-Rhododendron forest and grassy localities	32	40	15	2
D. heterocarpon (Linn) DC.	Suberect or prostrate under shrub	Upto 1600	Common in grassy localities and forest clearing	-	14	-	4
D. microphyllum (Thumb) DC	Diffused perennial herb	Upto 2000	Grassy localities, roadsides and forest edges	48	56	47	2
D. gangeticum Linn	Shrub	Upto 1500	Common in sal forest, roadsides grassy localities	45	47	38	3
Dolichos fulcatus Klein	Herb	1800-3200	In grassy localities	26	44	26	2
Erythrina arborescens Roxb.	Tree	1500-3000	Throughout the hills	45	56	55	3
Flemingia bracteata Wight.	Undershrub	1500	Common in sal forest	34	48	28	3
F. strobilifera R.Br.ex Ait	Shrub	Upto 2500	In oak forest and grassy slopes in chir pine forest	32	36	35	4
F. vestita Benth ex Baker	Trailing herb	1800-3000	Grassy localities throughout the hills	48	56	45	2
Indigofera cassioides Rottl. Ex DC	Shrub	300-1600	Common in sal, chir and miscellaneous forests	32	56	48	2
I. dosua Buch-Hm. Ex Don	Diffused shrub or under shrub	1300-3600	In open chir and oak forests	30	64	56	1
I. heterantha Wall ex Brandis	Shrub	Upto 3000	Throughout the hills along way sides and vacant plots in oak forest	60	72	50	1
Lathyrus aphaca Linn	Annual trailing herb	Upto 2200	Common in fields, roadsides and field borders	-	-	-	5
Lespedeza gerardiana Garh.ex Baker	Undershrub	1500-2000	Open grassy localities	35	47	40	2
Medicago denticulata Willd.	Annual procumbent herb	Upto 1500	Common in gardens, agricultural fields and waysides	-	-	-	5
M. lupulina Linn	Decumbent ascending herb	Upto 1500	Common in grassy localities and roadsides	26	32	12	4
Parochetus communis Buch.-Ham ex D. Don	Herb	Upto 3800	Common on damp shaded plces and grassy localities	45	55	50	3
Pterocarpus marsupium Roxb.	Tree	500-1300	In open miscellaneous forests	-	-	-	5
Smithia ciliata Royal	Diffused herb	800-1900	In marshy and sandy localities	25	54	28	3
Trifolium repens Linn	Perennial herb	1400-3200	In waste places, waysides and fields	47	58	36	3
Trigonella. emodi Benth	Herb	1500-3600	Common on grassy fields and ground vegetation in oak forest	47	58	50	3
T. gracilis Benth	Diffused herb	2000-3000	In forest edges and grassy slopes	35	40	20	2
Vicia pallida Turoz.	Climber	1000-2500	In waste places and agricultural fields	-	-	-	5

V. rigidula Royal	Herb	2000-2800	Forest edges, scrub jungles and roadsides	0	54	0	4
V. tetrasperma (Linn) Moench	Prostrate climbing annual herb	300-1800	Grassy localities	-	-	-	5
V.vexillata (Linn) A Richard	Trailing herb	Upto 2500	In pine forest and open grasslands	42	56	45	2
Zornia gibbosa Span.	Herb	Upto 1500	In grassy localities, river beds, roadsides and borders of agricultural field	36	42	40	3
Family Caesalpiniaceae							
Bahunia retusa Buch-Ham ex Roxb.	Tree	300-1800	Common in miscellaceous forests	48	56	-	4
B variegata Linn.	Tree	300-1900	In chir pine and miscellaceous forest	36	36	47	2
Caesalpinia bonduc (Linn) Roxb.	Rambling climber	Upto 1000	Common in miscellaneous forest	48	50	32	2
Cassia floribunda Cav.Descr.	Shruby herb	Upto 1800	Throughout the hills in waste lands	40	67	54	2
C. mimosoides Linn.	Prostrate herb	Upto 1600	In grassy open localities, roadside and wayside	38	36	28	2
C. occidentalis Linn	Erect herb or undershrub	Upto 1300	In waste lands, roadsides and sometimes near river banks	29	54	27	3
C. tora Linn.	Erect herb or undershrub	Upto 1200	In waste lands, roadsides and agricultural field	34	46	21	3
Delonix regia (Hook.) Raf.	Tree	Upto 1000	Planted roadside; and as ornamental plant	48	51	36	4
Family Mimosaceae							
Acacia farnesiana (Linn.) Willd	Shrub or small tree	Upto 1800	Throughout the area	47	55	15	3
A. nilotica (Linn) Del	Small tree	Upto 1400	In valleys	27	35	18	3
Albizia chinensis (Osbeck) Merr.	Tree	300-1400	Scattered in open and miscellaneous forests	40	46	24	3
Albezia lebbeck (Linn.) Willd	Large tree	Upto 1500	In miscellaneous forests	43	56	42	2
Leucaena leucocephala (Lam.) De. Wit.	Tree	Upto 1400	Planted	26	48	25	2
Mimosa himalayana Gamble	Small tree or straggling shrub	Upto 1600	Along the water courses and scrub jungles	46	58	42	1
M. pudica Linn.	Under shrub	Upto 1500	Common on roadsides and waste places	37	40	12	3

A =percent external colonization; B= percent internal colonization C= percent vesicles

- 1= Excellent; 2= Good; 3= Moderate; 4= Poor; 5= Nil

4. Discussions

Degradation of natural resource and environment has been a global problem. In India, more than half of its geographical area faces problem of land degradation of one or the other kind. In the Himalayan region, degradation of forests has become a wide spread feature and restoration of such degraded land has become a major challenging conservational problem. Many efforts have been made to check the natural resource but have not yielded the desired sustainability. Microbial populations are key components of the soil-plant system where they are immersed in a framework of interactions affecting plant development (Lynch 1990).Perhaps the most widespread and certainly significant mutualism between plants and fungi is the root symbiosis, termed as arbuscular mycorrhiza(AM). These AM fungi are the most common natural association makers with the nodulated nitrogen-fixing legumes and other plants (Kothamasi et al 2001).

Woody legumes are useful for revegetation of water- deficient ecosystems that have low availability of N, P and other nutrients (Danso 1992). The scarcity of available P and the imbalance of trace elements in degraded ecosystems actually limit establishment of legumes and nitrogen fixation. But when associated with mycorrhizae it was found to increase the establishment of legumes (Barea et al 1992). In addition, woody legumes exhibit a considerable degree of dependence on mycorrhizae to thrive in stressed conditions (Osonubi et al 1991). After forming symbiotic association with legume roots, AM fungi develops an extraradical mycelium that links the

roots and soil environment and help the plants to use soil nutrients more efficiently (Barea et al 1992).

Sustainable management of any degraded ecosystem involves practices that are equally concerned with productivity and soil conservation. In this situation, the Vesicular-arbuscular mycorrhizal legumes could reduce the amount of fertilizer needed for the establishment of vegetation and also increase the rate at which the desired vegetation becomes established by stimulating the development of beneficial microorganisms in the rhizosphere. Since, the cell walls of VAM hyphae are composed of amino-sugar chitin, the soil mycelium may be one of the most important vehicles for nitrogen and carbon input into the soil. Owing to their role, the VAM legumes can be used for revegetation of eroded, desertified or degraded ecosystems. In addition, the dual inoculation (Vesicular- Arbuscular Mycorrhiza along with *Rhizobium*) can be a biological tool for the management of N_2- fixing plants in restoring and maintaining soil fertility.

The capacity of VAM fungi to act as biofertilizers, bioregulators and bioprotectors has repeatedly been demonstrated. These associations help to maintain the general plant vigour under a variety of adverse and inhospitable ecological conditions. Present investigation is an attempt to enhance our knowledge of the ecology and applicability of VA Mycorrhizal legumes in successful reclamation and restoration practices in degraded ecosystems. On degraded sites where original soil has deteriorated markedly, these VAM associated nitrogen- fixing species can be planted. They can improve soil condition and growth of associated plant species through nutrient-rich leaf litter and biological nitrogen-fixation and therefore, useful in restoration of degraded lands.

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Model for Calculating the Concentration of Dissolved Iron Relative to the Final Solution pH and Temperature during Oxalic Acid Leaching of Iron Oxide Ore.

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Abstract: Model for calculating the concentration of dissolved iron (relative to the final solution pH and temperature) during leaching of iron oxide ore in oxalic acid solution has been derived. The model;

$$\%Fe = 1.1849(\gamma/T)^3$$

was found to calculate the concentration of dissolved iron being dependent on the values of the final leaching solution pH and temperature measured during the leaching process. It was observed that the validity of the model is rooted in the expression $(\%Fe/N)^{1/3} = \gamma/T$ where both sides of the expression are approximately equal to 0.2. The maximum deviation of the model-predicted concentration of dissolved iron from the corresponding experimental values was found to be less than 18% which is quite within the acceptable range of deviation limit of experimental results. Concentrations of dissolved iron per unit rise in the solution temperature as obtained from experiment and derived model were evaluated as 0.0011 and 0.0015 $\%/^0C$ respectively, indicating proximate agreement. [Journal of American Science. 2011;7(1):12-18]. (ISSN: 1545-1003).

Keywords: Model, Dissolved Iron, Solution pH and Temperature, Oxalic Acid, Iron Oxide Ore.

1. Introduction

The mixed potential model of leaching assumes that the charge transfer processes occurring at the mineral surfaces are those that control the rate of dissolution (Kanevskii et al, 1963).

Nwoye et al. (2009a) derived a model for predicting the initial solution pH at assumed final pH and leaching time during leaching of iron oxide ore in hydrogen peroxide solution. It was observed that the validity of the model is rooted in the mathematical expression; $(\ln t)^{1/2} = N(\beta^C/\alpha^C)$ where both sides of the relationship are approximately equal to 2. The model is expressed as;

$$\beta = \text{Antilog}[0.2439\text{Log}(\alpha^{4.1}(\ln t)^{1/2}/3.6)] \qquad (1)$$

The model shows that the initial solution pH is dependent on the values of the final solution pH and leaching time. The respective positive or negative deviation of the model-predicted final pH from its corresponding experimental value was found to be less than 8%, which is quite within the acceptable deviation limit of experimental results depicting the validity of the model.

Model for calculating the solution pH during hydrogen peroxide leaching of iron oxide ore has also been derived by Nwoye et al. (2009b). It was observed that the validity of the model is rooted in the expression $\ln\gamma = K_C[(\%Fe_2O_3/\%Fe)^N]$ where both sides of the equation are correspondingly approximately equal to 2. The model is expressed as;

$$\gamma = \exp\left(K_C[(\%Fe_2O_3/\%Fe)^N]\right) \qquad (2)$$

The final solution pH was found to depend on the values of the % concentrations of dissolved iron and haematite from experiment. The respective deviation of the model-predicted pH values from the corresponding experimental values was found to be less than 20% which is quite within the acceptable range of deviation limit of experimental results.

Nwoye et al. (2008) derived a model for evaluation of the concentration of dissolved iron (relative to the final solution pH and temperature) during leaching of iron oxide ore in sulphuric acid solution. It was observed that the validity of the model was rooted in expression $(\%Fe/N)^{1/3} = \alpha/T$ where both sides of the expression are approximately equal to 0.2. The model is expressed as;

$$\%Fe = 0.35(\alpha/T)^3 \qquad (3)$$

Where

T = Solution temperature at the time t when the concentration of dissolved iron is

evaluated. (^{0}C)

N= 0.35(pH coefficient for sulphuric acid solution during leaching of iron oxide ore) determined in the experiment (Nwoye, 2007).

α = Final pH of the leaching solution at the time t when the concentration of dissolved iron is evaluated.

Model for evaluation of the concentration of dissolved phosphorus (relative to the final pH of the leaching solution) during leaching of iron oxide ore in oxalic acid solution has been derived by Nwoye (2009). The model is expressed as;

$$P = e^{(12.25/\alpha)} \qquad (4)$$

Where

P = Concentration of phosphorus removed during the leaching process (mg/Kg)

N = 12.25; (pH coefficient for phosphorus dissolution in oxalic acid solution) determined in the experiment (Nwoye, 2003).

α = Final pH of the leaching solution at the time t when the concentration of dissolved phosphorus is evaluated.

It was observed that the validity of the model is rooted in the relationship $\ln P = N/\alpha$ where both sides of the expression are approximately equal to 4. The model depends on the value of the final pH of the leaching solution which varies with leaching time. In all, the positive or negative deviation of the model-predicted phosphorus concentration from its corresponding value obtained from the experiment was found to be less than 22%, which is quite within the acceptable deviation limit of experimental results hence establishing the validity and precision of the model.

Model for quantitative analysis of dissolved haematite (relative to the initial solution pH) during leaching of iron oxide ore in oxalic acid solution has been derived by Nwoye et al. (2009c). The model;

$$\%Fe_2O_3 = \left(\frac{N}{N_c}\left(\frac{1}{\gamma}\right)\right) \qquad (5)$$

was found to calculate the concentration of dissolved haematite being dependent on the values of the initial leaching solution pH measured during the leaching process. The respective positive and negative deviation of the model-predicted values of $\%Fe_2O_3$ (dissolved) from the corresponding experimental values was found to be less than 11% which is quite within the acceptable range of deviation limit of experimental results. The values of the assumed coefficients of dilution (N) and dissolution of haematite (N_c) in oxalic acid solution were calculated to be 197.7503 and 700.0618 respectively.

Nwoye (2008a) derived a model for evaluating the final pH of the leaching solution during leaching of iron oxide ore in oxalic acid solution. The model evaluates the pH value as the sum of two parts, involving the % concentrations of Fe and Fe_2O_3 dissolved. The model can be expressed as;

$$\gamma = 0.5\left(\frac{K_1}{\%Fe} + \frac{K_2}{\%\ Fe_2O_3}\right) \qquad (6)$$

Where

K_1 and K_2 = dissolution constants of Fe and Fe_2O_3 respectively.

γ = final pH of leaching solution (after time t).

It was also found that the model (Nwoye, 2008a) could predict the concentration of Fe or Fe_2O_3 dissolved in the oxalic acid solution at a particular final solution pH by taking Fe or Fe_2O_3 as the subject formular. The prevailing process conditions under which the model works include: leaching time of 30mins., constant leaching temperature of 30^{o}C, average ore grain size; 150μm and 0.1M oxalic acid.

Nwoye (2008b) has reported that the heat absorbed by oxalic acid solution during leaching of iron oxide ore can be predicted using the derived model which works under the process condition; initial pH 6.9, average ore grain size; 150μm and leaching temperature; 30^{0}C. The model (Nwoye, 2008b) can be stated as

$$Q = K_N\left(\frac{\gamma}{\%Fe_2O_3}\right) \qquad (7)$$

Where

Q = Quantity of heat absorbed by oxalic acid solution during the leaching process. (J)

γ = Final pH of the leaching solution (at time t).

$\%Fe_2O_3$= Concentration of haematite dissolved in oxalic acid solution during the leaching process.

K_N = 4.57(Haematite dissolution constant in oxalic acid solution) determined in the experiment (Nwoye, 2008c).

Nwoye (2008b) carried out further work on the model using the same process conditions and observed that on re-arranging the model as;

$$\%Fe_2O_3 = K_N\left(\frac{\gamma}{Q}\right) \qquad (8)$$

the concentrations of haematite predicted deviated very insignificantly from the corresponding experimental values. In this case, the value of Q was calculated by considering the specific heat capacity of oxalic acid. Values of heat absorbed by the oxalic acid solution during the leaching of iron oxide ore as predicted by the model (Nwoye, 2008b) agree with the experimental values that the leaching process is endothermic. This is because all the predicted values of the heat absorbed by the oxalic acid solution were positive. The model shows that the quantity of heat absorbed by oxalic acid solution during the leaching process is directly proportional to the final pH of the solution and inversely proportional to the concentration of haematite dissolved.

Nwoye et al. (2009d) derived a model for calculating the concentration of leached iron during leaching of iron oxide ore in sulphuric acid solution. The model is expressed as;

$$\%Fe = e^{-2.0421(\ln T)} \qquad (9)$$

The model was found to predict %Fe (leached) very close to the values obtained from the experiment, being dependent on the values of the final leaching solution temperature measured during the leaching process. It was observed that the validity of the model is rooted in the expression ln(%Fe) = N(InT) where both sides of the expression are correspondingly approximately equal. The positive or negative deviation of each of the model-predicted values of %Fe (leached) from those of the experimental values was found to be less than 37%.

Model for predictive analysis of the concentration of dissolved iron during leaching of iron oxide ore in sulphuric acid solution was derived by Nwoye et al. (2009). The model expressed as;

$$\%Fe = 0.987(\mu/T) \qquad (10)$$

was found to predict %Fe dissolved with high degree of precision being dependent on the values of the leaching temperature and weight of iron oxide ore added. It was observed that the validity of the model is rooted in the expression %Fe = N(μ/T) where both sides of the relationship are correspondingly approximately equal. The positive or negative deviation of each of the model-predicted values of %Fe (dissolved) from those of the experimental values was found to be less than 19% which is quite within the acceptable range of deviation limit for experimental results, hence depicting the usefulness of the model as a tool for predictive analysis of the dissolved iron during the process.

Nwoye (2010) derived a model for computational analysis of the solution temperature (relative to the final pH of the leaching solution) during leaching of iron oxide ore in oxalic acid solution. The model;

$$T = e^{(14.9661/p)} \qquad (11)$$

shows that the solution temperature is dependent on the value of the final pH of the leaching solution which varies with leaching time. It was observed that the validity of the model is rooted on the expression $\ln T = K_c/p$ where both sides of the equation are approximately equal to 3. The maximum deviation of the model-predicted solution temperature values from those of the experimental values were found to be less than 9% hence establishing the validity and precision of the model. The correlation between mass of iron oxide ore and solution temperature as well as between final pH of leaching solution and solution temperature as obtained from experiment and derived model (0.9296 and 0.8911 as well as 0.9395 and 0.9988) respectively are quite close, indicating proximate agreement with values from actual experiment.

The aim of this work is to derive a model for calculating the concentration of dissolved iron relative to the final solution pH and temperature during leaching of Agbaja (Nigeria) iron oxide ore in oxalic acid solution.

2. Model

The solid phase (ore) is assumed to be stationary, contains the un-leached iron remaining in the ore. Hydrogen ions from the oxalic acid attack the ore within the liquid phase in the presence of oxygen.

2.1 Model Formulation

Experimental data obtained from research work (Nwoye, 2006) carried out at SynchroWell Research Laboratory, Enugu were used for this work. Results of the experiment as presented in report (Nwoye, 2006) and used for the model formulation are as shown in Table 1.

Computational analysis of the experimental data (Nwoye, 2006) shown in Table 1, resulted to Table 2 which indicate that;

$$\left(\frac{\%Fe}{N}\right)^{1/3} = \left(\frac{\gamma}{T}\right) \quad \text{(approximately)} \qquad (12)$$

$$\%Fe = N\left(\frac{\gamma}{T}\right)^3 \qquad (13)$$

Introducing the value of N into equation (13)

$$\%Fe = 1.1849\left(\frac{\gamma}{T}\right)^3 \qquad (14)$$

Where

T= Solution temperature during leaching of iron oxide ore using oxalic acid (^{0}C)

N= 1.1849 (pH coefficient for iron dissolution in oxalic acid solution during leaching of iron oxide ore) determined in the experiment (Nwoye,2006).

γ = Final pH of the leaching solution at the time t when the concentration of dissolved iron is evaluated.

Equation (14) is the derived model**.**

Table 1: Variation of dissolved iron with final solution pH and temperature. (Nwoye,2006)

(γ)	T (^{0}C)	%Fe
4.33	25.10	0.0074
4.43	25.30	0.0063
4.48	25.50	0.0055
4.64	25.70	0.0074
4.84	26.10	0.0085

3. Boundary and Initial Condition

Consider iron ore in cylindrical flask 30cm high containing leaching solution of oxalic acid. The leaching solution is stationary i.e (non-flowing). The flask is assumed to be initially free of attach bacteria. Initially, atmospheric levels of oxygen are assumed. Range of mass of iron oxide ore was used: (6-20g). The initial pH of leaching solution; 4.5 and leaching time of 30 minutes was used for all samples. A constant leaching temperature of 25^{o}C was used. Ore grain size; 150μm, volume of leaching solution; 0.1litre and oxalic acid concentration; 0.1mol/litre were used. These and other process conditions are as stated in the experimental technique (Nwoye, 2006). The boundary conditions are: atmospheric levels of oxygen (since the cylinder was open at the top) at the top and bottom of the ore particles in the liquid and gas phases respectively. At the bottom of the particles, a zero gradient for the liquid scalar are assumed and also for the gas phase at the top of the particles. The leaching solution is stationary. The sides of the particles are taken to be symmetries.

4. Model Validation

The formulated model was validated by direct analysis and comparison of %Fe values from model data and those from the experimental data for equality or near equality. Analysis and comparison between these data reveal deviations of model data from experimental data. This is believed to be due to the fact that the surface properties of the ore and the physiochemical interactions between the ore and leaching solution which were found to have played vital roles during the leaching process (Nwoye, 2006) were not considered during the model formulation. This necessitated the introduction of correction factor, to bring the model data to that of the experimental values. (Table 3)

Deviation (Dv) of model predicted %Fe values from experimental %Fe values is given by

$$Dv = \left(\frac{Dp - DE}{DE}\right) \times 100 \qquad (15)$$

Where Dp = Predicted data from model

DE = Experimental data

Correction factor (Cf) is the negative of the deviation i.e

$$Cf = -Dv \qquad (16)$$

Therefore

$$Cf = -100\left(\frac{Dp - DE}{DE}\right) \qquad (17)$$

Introduction of the corresponding values of Cf from equation (17) into the model gives exactly the corresponding experimental %Fe values (Nwoye, 2006).

5. Results and Discussion

The derived model is equation (14). Computational analysis of values in Table 1 resulted to Table 2.

Table 2: Variation of $(\%Fe/N)^{1/3}$ with γ/T

$(\%Fe/N)^{1/3}$	γ/T
0.1842	0.1725
0.1745	0.1751
0.1668	0.1757
0.1842	0.1805
0.1929	0.1854

An ideal comparison of the %Fe values as obtained from experiment (Nwoye, 2006) and as predicted by the model for the purpose of testing the validity of the model is achieved by considering the R^2 values (coefficient of determination). The values of the correlation coefficient, R calculated from the equation;

$$R = \sqrt{R^2} \qquad (18)$$

using the r-squared values (coefficient of determination) from Figures 1-4. Comparison between these figures shows a better correlation between final solution temperature and dissolved iron as well as between final solution pH and dissolved iron for

derived model (0.9748 and 0.9946) compared to that obtained from experiment (Nwoye, 2006) (0.8590 and 0.8574).

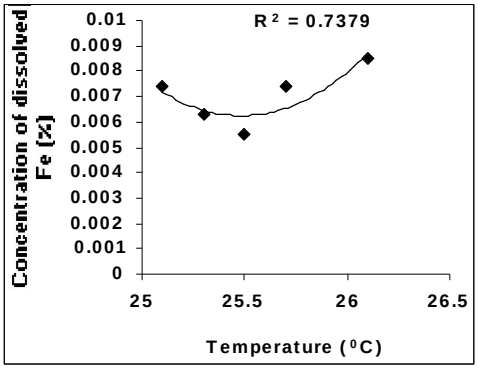

Figure 1- Effect of solution temperature on the concentration of dissolved iron (as obtained from experiment (Nwoye, 2006))

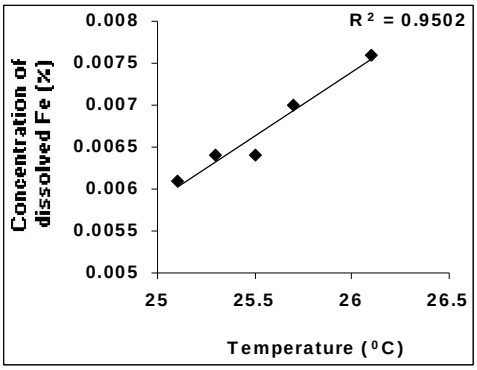

Figure 2- Effect of solution temperature on the concentration of dissolved iron (as predicted by derived model).

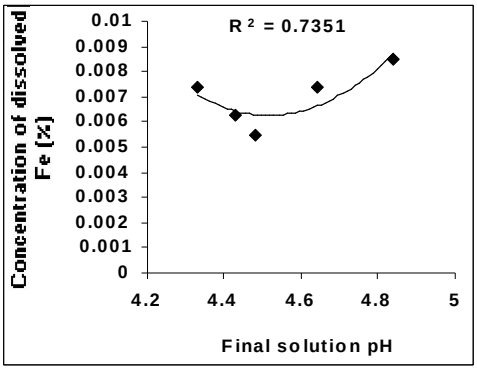

Figure 3- Effect of final solution pH on the concentration of dissolved iron (as obtained from experiment (Nwoye, 2006))

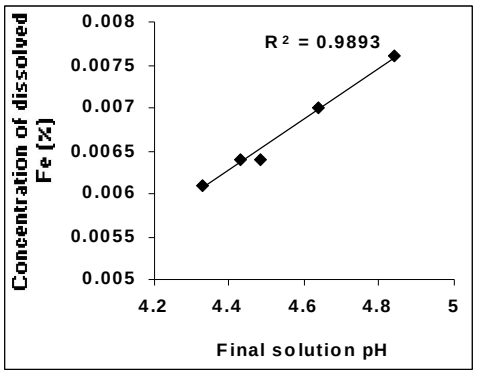

Figure 4- Effect of final solution pH on the concentration of dissolved iron (as predicted by derived model).

Figures 5 and 6 show very close alignment of the curves from model-predicted values (line MoD) and that from the corresponding experimental values (line ExD). The degree of alignment of these curves is indicative of the proximate agreement between both experimental and model-predicted values of dissolved iron. The model predicts (from Figures 2 and 4) that the final solution pH plays a better role (than final solution temperature) towards enhancing the concentration of dissolved iron. However, experimental results (Nwoye, 2006) as shown in Figures 1 and 3 indicate that final solution temperature plays a better role.

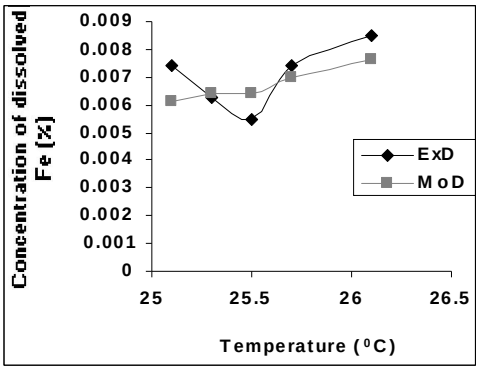

Figure 5- Comparison of the concentrations of dissolved iron relative to the final solution temperature as obtained from experiment (Nwoye, 2006) and derived model

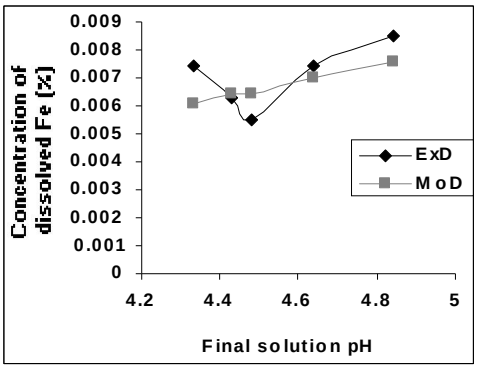

Figure 6- Comparison of the concentrations of dissolved iron relative to the final solution pH as obtained from experiment (Nwoye, 2006) and derived model

The concentration of dissolved iron per unit rise in the solution temperature during the leaching process was determined following comparison of the concentration of dissolved iron per unit rise in the solution temperature obtained by calculations involving experimental results as well as derived model.

Determination of the concentration of dissolved iron per unit rise in the solution temperature

Concentration of dissolved iron during leaching in oxalic acid solution per unit rise in the solution temperature I_T (%/^{0}C) is calculated from the equation;

$$I_T = I/T \qquad (19)$$

Therefore, a plot of concentration of dissolved iron against solution temperature (as in Figure 1) gives a slope, S at points (0.0074, 25.1) and (0.0085, 26.1) following their substitution into the mathematical expression;

$$S = \Delta I/\Delta T \qquad (20)$$

Eqn. (20) is detailed as

$$S = I_2 - I_1/ T_2 - T_1 \qquad (21)$$

Where

ΔI = Change in the concentrations of iron dissolved I_2, I_1 at solution temperature values T_2, T_1. Considering the points (0.0074, 25.1) and (0.0085, 26.1) for (I_1, T_1) and (I_2, T_2) respectively, and substituting them into eqn. (21), gives the slope as 0.0011 %/^{0}C which is the concentration of dissolved iron per unit rise in the solution temperature during the actual experimental (Nwoye, 2006) leaching process. Also similar plot (as in Figure 2) of model-predicted results gives a slope. Considering points (0.0061, 25.1) and (0.0076, 26.1) for (I_1, T_1) and (I_2, T_2) respectively and substituting them into eqn. (21) gives the value of slope, S as 0.0015 %/^{0}C. This is the model-predicted concentration of dissolved iron per unit rise in the solution temperature. A comparison of these two values of dissolved iron concentrations per unit rise in the final solution temperature shows proximate agreement.

Table 3 shows that the maximum deviation of the model-predicted values of %Fe from the corresponding experimental values (Nwoye, 2006) is less than 18% which is quite within the acceptable range of deviation limit of experimental results hence depicting the reliability and validity of the model. The validity of the model is believed to be rooted on equation (12) where both sides of the equation are approximately equal to 0.2. Table 2 also agrees with equation (12) following the values of $(\%Fe/N)^{1/3}$ and γ/T evaluated from Table 1 as a result of computational and statistical analysis.

Table:3 Variation of model-predicted concentration of dissolved iron with the associated deviation and correction factors

%Fe	Dv(%)	Cv (%)
0.0061	-17.57	+17.57
0.0064	+1.59	-1.59
0.0064	+16.36	-16.36
0.0070	-5.41	+5.41
0.0076	-10.59	+10.59

The least and highest magnitude of deviation of the model-predicted %Fe (from the corresponding experimental values) are + 1.59% and -17.57% which correspond to solution temperatures 25.3 and 25.1^{0}C respectively. Table 3 indicates that a correction factor of -1.59 % and + 17.57% make up for the least and highest deviation of +1.59% and -17.57% resulting from final solution pH of 4.43 and 4.33 and solution temperature: solution temperatures 25.3 and 25.1^{0}C. It is pertinent to state that the actual deviations are just the modulus of the values. The role of the sign attached to the values is just to show when the deviation is surplus or deficit.

6. Conclusion

The model computes the concentration of dissolved iron relative to the final solution pH and temperature during leaching of Agbaja iron oxide ore. The validity of the model is believed to be rooted in the expression $(\%Fe/N)^{1/3} = \gamma/T$ where both sides of the expression are approximately equal to 0.2. The maximum deviation of the model-predicted values of %Fe from the corresponding experimental values is less than 18% which is quite within the acceptable range of deviation limit of experimental results. The concentrations of dissolved iron per unit rise in the solution temperature as obtained from experiment and derived model were evaluated as 0.0011 and

0.0015 %/^{0}C respectively, indicating proximate agreement.

Further works should incorporate more process parameters into the model with the aim of reducing the deviations of the model data from that of the experimental.

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Cytogenetic effect of Insecticide Telliton and Fungicide Dithane M-45 on Meiotic Cells and Seed Storage Proteins of *Vicia faba*.

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Abstract: The genotoxic effects of insecticide Telliton and fungicide Dithane M-45 were examined on meiotic cell divisions and changes in the M2 seed storage protein banding pattern of *Vicia faba* plants. The percentage of abnormal pollen mother cells, (PMCs) increased as the concentration of both pesticides increased. All concentrations and treatment periods of both pesticides, induced a number of chromosomal aberrations in PMCs as stickiness, bridges, laggards, disturbed, micronuclei and multinucleate. A marked change was observed in the M2 *V. faba* seed storage protein banding pattern. These changes included alterations in band intensity, relative mobilities, disappearance of some bands and appearance of new other ones. These results showed that Telliton has more mutagenic effects than Dithane M-45. [Journal of American Science. 2011;7(1):19-25]. (ISSN: 1545-1003).

Key words: *Vicia faba*, chromosomal abnormalities, insecticide, fungicide and SDS -PAGE protein.

1. Introduction:

Pesticides are used all over the world, their use has increased spectacularly because it has greatly improved agricultural yield through inhibition of diseases by acting against pests in the field and during storage of agricultural products Taylor *et al.*, (1997). A number of pesticides are used to protect agricultural products from diseases, weeds and insects, but residues of these chemicals lead to environmental pollution and pose threat to people and animals. Although chemical control creates several problems, use of pesticides is still maintaining its popularity for obtaining effective results. Now, After increased application of many new agrochemicals on a large scale in the egyptian agriculture and other countries has led some workers to investigate the possible genetic material and storage proteins alterations Badr (1988), Abdel Salam *et al*., (1993a & b), Hassan (1996), George and Ghareeb (2001) Asita and Makhalemele (2009). Telliton known as profenophos is the insecticide commonly used in delta Egypt region in agricultural fields. Dithane M-45, also known as mancozed fungicide belongs to a class of chemicals as ethylene bisdithiocarbamate (EBDC).The EBDC is fungicide used to prevent crop damage in the field and to protect harvested crops from deterioration during storage or transport. Chromosomal aberrations have been considered as a reliable indicator of mutagenic activity, since there have been evidence for a correlation between chromosomal damage and toxic effects of a number of pesticides Badr (1983), Askin (2006), Shehata *et al*., (2008), Ozturk (2008) and Fisun and Goc Rasgele (2009). On the other hand, Abdelsalam *et al.*, (1993b) and Hassan *et al*., (2002) used electrophoretic banding patterns of seed storage proteins for monitoring the mutagenic effects of pesticides and other chemicals.

The present work was planned to study the mutagenic effect of the insecticide Telliton and fungicide Dithane M-45 as revealed by meiotic abnormalities and changes in M2 seed storage protein banding patterns of *V. faba* as a biological system.

2. Materials and Methods:

Vicia faba L. variety Giza 3, kindly procured from Crop Research Institute, Agricultural Research Center, Giza, Egypt.

1- Meiosis

Vicia faba plants at the flowering stage were sprayed with different concentrations of the insecticide Telliton, (*0*-4-bromo-2-chlorophenyl *0*-ethyl s-propyl phosphorothioate) 1.5, 3, and 6 ml/L and the fungicide Dithane M-45(ethylene-bis dithiocarbamate) 150, 300 and 600 mg/L. These, pesticides selection were purely on the basis of the frequent use in the agricultural fields by the farmers of Delta, Egypt. A negative control plants were sprayed with distilled water. Eight flower buds from eight different plants were gathered through durations of 24 h., 48 h. and 10 days.

For meiotic studies the appropriate flower buds were collected and fixed in Carnoy's solution (ethyl alcohol absolute and glacial acetic acid in the ratio 3:1) for 24 h. and then transferred to 70% ethyl alcohol and kept in refrigerator. The cytological analysis were carried out by using 2% aceto-carmine stain as described by Darlington and La Cour(1976). The data recorded for different treatments were statistically analyzed using *t*-test for determine significant differences between these treatments.

2-Electrophoresis of water soluble and non soluble proteins:

Water soluble and non soluble proteins were performed on vertical slab (20 cm x 20 cm x 0.2 cm) using the gel electrophoresis apparatus (Manufactured by LABCONCO) according to Laemmli (1970). The dry M2 seeds of *V. faba* plants, whose parents were sprayed with these pesticides, were decoated and milled to fine powder. Soluble proteins were extracted overnight using 0X Tris-Hcl buffer of pH 6.8. Centrifugation was performed at 10000 rpm for 10 min., then the non soluble proteins were extracted from the belt by add IX Tris-Hcl buffer pH 6.8 for 24 h. and then centrifuged at 10000 rpm for 10 min., then 40 µl supernatant of soluble and non-soluble proteins were loaded in SDS-slab gel of 15% acrylamide containing 10% SDS. Gel was run at a current of 15 mA for 1 hour followed by 25 mA for 4-5 h. Molecular weights of different bands were calibrated using the wide range protein marker ranged from 10 -200 KDa according to Matta *et al.*, (1981).

3. Results and Discussion:

I-Cytological studies:

A wide spectrum of chromosomal abnormalities were recorded in eight flower buds from different plants after treatment with different concentrations of Telliton (1.5, 3 & 6 ml/L) and Dithane (150, 300 & 600 mg/L). The number of meiotic cells of treated and control plants are presented in Tables (1& 2). The insecticide Telliton give the number of chromosomal abnormalities higher than fungicide Dithane. Both pesticides caused a hollow range of meiotic abnormalities. The number of abnormal pollen mother cells (PMCs) formed in the flower buds of *V. faba* plants was obvious with all concentrations of pesticides and in all stages and durations.

Data in Tables 1& 2 shows that the percentages of abnormal PMCs in the first division were greater than those recorded in the second division after spraying with both pesticides. The most frequent types of abnormalities were observed stickiness, laggards, bridges, disturbed, micronuclei and multinuclei after being treated with all concentrations of both pesticides. These results demonstrated in Tables (1&2) and Fig.1 revealed that the abnormalities were present in metaphase, anaphase and telophase stages of the meiosis with all treatments. The induction of meiotic abnormalities appears to be a common effect of most pesticides (Fisun & Goc Rasgele, 2009).

The stickiness and disturbed stages were the most common abnormalities found in all phases of the meiosis after treatments with all doses of both pesticides (Fig.1). The number of sticky cells increased in all stages of meiotic divisions as the concentration of both pesticides increased during durations of 24 h, 48 h and 10 days. Our results are in agreement with the results of Badr (1988); Pandey *et al.*, (1994); Singh *et al.* (2007) and Srivastava & Singh (2009). Abdelsalam *et al.*, (1993b) they suggested that the chromosome stickiness may results from breakage and exchange between chromatin fibers on the surface of adjoining chromosomes.

The second type of abnormalities is the laggard that occurred at metaphase cells. They could be attributed to the failure of the spindle apparatus to organize and function in a normal way Pickett-Heaps *et al*., (1982). These laggards may be distributed randomly to either poles at anaphase I or II which result ultimately in aneuploidy (Amer & Mikhael, 1987; Amer & Ali, 1988) or may give for micronuclei at telophase II (Abdelsalam *et al*., 1993 a). The induction of laggard chromosomes could be attributed to irregular orientation of chromosomes (Patil and Bhat, 1992).

In addition to the previous common abnormalities, it was observed more on meiotic division including bridges, micronuclei and multinucleate. Bridges were induced under the treatment with both pesticides. They could be due to the breakage and reunion (El-Khodary *et al*., 1990) or due to the general stickiness of chromosomes (Haliem, 1990).While, micronuclei and multinucleate were also recorded with low percentages after treatment with both pesticides and our results are in agreement with the results of Badr (1988) and Pandey *et al.*, (1994). Finally, the induction of these chromosomal abnormalities were pointed to the mutagenic potential of the applied concentrations of these pesticides.

II-Biochemical studies:

At the biochemical genetic level, water soluble and non-soluble protein, Table (3 & 4) and Fig. (2) represent the mutagenic effects of both pesticides, Telliton and Dithane on the banding pattern of M2 seed storage proteins of *V. faba* plant. These changes include alterations in band intensity, relative mobilities, disappearance of some bands and appearance of some new other bands.

Alterations in bands intensity could be attributed to change in the structure or performance of genes and thus they produce changes in the gene expression of the regulator genes used in the regulatory system of the structural genes Hassan 1996. The increase in band(s) intensity could be attributed to gene(s) duplication that resulted from cytological abnormalities induced by applied pesticides. The presence of laggards and bridges support this conclusion. This conclusion is in agreement with

Gamal El-Din *et al*., (1988).Also, they noticed that increasing the number of genes encoding for the different protein subunits through doubling of chromosome number from 12 to 24 in *V. faba* caused an increase in band intensity.

Changes in relative mobility of these bands are probably due to point mutation that leads to production of shorter or longer polypeptide chains. These changes in the soluble proteins are probably due to the occurrence of gene duplication mutation more than point mutation that takes place in one or more of the duplicated genes that encoding the protein subunit of that band Abdelsalam *et al*., (1993b). Also, these alterations in bands intensities or densities and relative mobility are in agreements with Hassan (1996), George and Ghareeb (2001) and Hassan *et al*., (2002).

The disappearance of some bands in soluble and non soluble proteins of *V. faba* to the inherited effects of the both pesticides, Telliton and Dithane could be explained on the basis of mutational event at the regulatory genes that prevent or attenuate transcription (Muller & Gottschalk, 1973).Induction of laggards, bridges and micronuclei by these pesticides may lead to the loss of genetic materials. Therefore, some electrophoretic bands were disappeared due to the loss of their corresponding genes (Abdelsalam *et al.,* 1993b). They also reported that the reduction of chromosome complement in *V. sativa* (2n=6) lead to the complete disappearance of the convicilin like band. The present results therefore may point out a mutagenic potential of both pesticides, Telliton and Dithane as indicated by observing a large number of the meiotic abnormalities and the heritable changes in the M2 seed storage protein banding patterns.

Table (1): Numbers and percentages of abnormal PMCs in the1st & 2nd meiotic divisions, percentages of types and mean of meiotic abnormalities after spraying of *V.faba* plants with Telliton insecticide for (24, 48 hours & 15 days).

Time	Conc. In Ml/L	No. of counted PMCs	No. of abnormal PMCs	% abnormal In 1st division	% abnormal In 2nd division	Types and percentages of meiotic abnormalities						Mean of % abnormal PMCs ± SE
						Stick.	Lag	Brid.	Dist.	Micronuclei	Multinuclei	
24 h	Cont.	7684	12.00	0.19	0.11	25.00	16.66	8..33	50.00	-	-	0.16±0.03
	1.5	5761	479	8.15	8.49	29.23	13.36	7.72	47.59	0.83	1.25	8.31±1.04
	3	4677	718	18.14	12.68	35.93	12.39	6.82	42.06	1.11	1.67	15.35±1.32
	6	3471	924	28.43	24.89	42.09	11.04	6.17	35.60	1.62	3.46	26.62±1.12
48h	1.5	3023	567	10.55	8.20	31.75	13.76	7.41	43.56	1.59	1.94	9.38±1.12
	3	2968	656	13.00	9.10	31.09	14.18	7.32	42.84	1.98	2.59	11.05±1.22
	6	2741	798	17.10	12.01	35.34	14.16	6.52	39.59	1.50	2.88	14.55±1.34
10 days	1.5	4672	390	4.64	3.70	28.97	12.05	7.43	50.25	0.51	0.77	4.17±0.94
	3	4195	506	7.44	4.67	33.00	13.04	6.32	44.66	1.78	1.19	6.06±1.01
	6	3841	565	8.25	6.46	35.75	12.92	6.55	41.42	1.42	1.95	7.36±1.32

Stick: stickiness Lag.: laggards Brid.: bridge Dist.: disturbed PMCs : pollen mother cells

Table (2): Numbers and percentages of abnormal PMCs in the1st & 2nd meiotic divisions, percentages of types andmean of meiotic abnormalities after spraying *V. faba* plants with Dithane fungicide for (24, 48 hours & 15 days).

Time	Conc. In mg/L	No. of counted PMCs	No. of abnormal PMCs	% Abnormal In 1st division	% Abnormal In 2nd division	Types and percentages of meiotic abnormalities						Mean of % abnormal PMCs ± SE
						Stick.	Lag	Brid.	Dist.	Micronuclei	Multinuclei	
24 hr	Cont.	7684	12.00	0.19	0.11	25.00	16.66	8..33	50.00	-	-	
	150	6142	384	3.58	2.67	25.26	14.06	10.67	47.14	0.52	1.82	0.16±0.03
	300	5639	463	4.58	3.64	24.62	16.41	11.66	43.84	1.08	2.37	3.12±0.90
	600	5173	628	6.57	5.56	29.30	18.78	11.46	36.15	1.91	2.39	4.11±1.10
48hr	150	5672	298	2.96	2.29	26.17	14.42	12.08	43.28	1.67	2.35	6.07±1.20
	300	5113	315	3.23	2.93	26.66	16.50	13.33	40.63	0.95	1.90	2.63±0.80
	600	4370	362	4.53	3.75	26.79	16.85	13.81	37.56	1.65	4.42	3.08±1.02
10 days	150	6176	256	2.14	2.01	23.04	14.84	12.11	45.31	0.78	3.91	4.14±1.32
	300	6214	291	2.43	2.25	24.39	15.81	13.05	41.24	1.72	3.78	2.07±1.00
	600	5365	310	3.31	2.46	26.45	16.45	15.81	36.77	1.94	2.58	2.34±1.36

Stick: stickiness, Lag.: laggards, Brid.: bridge, Dist: disturbed, PMCs : pollen mother cells.

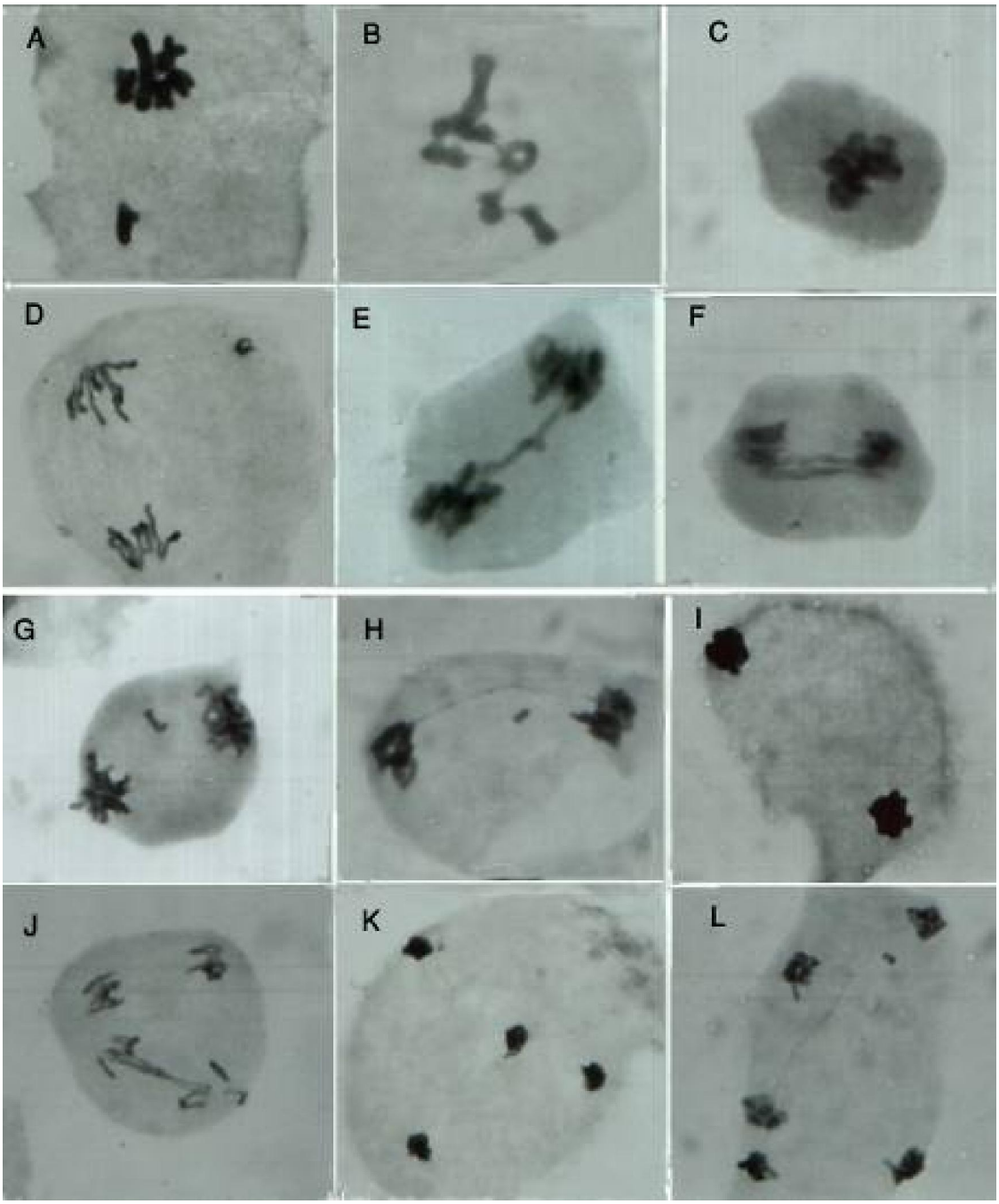

Fig (1): Types of chromosomal abnormalities produced after treatments with different concentrations of Telliton and Dithane. A- metaphase I with laggard. B- metaphase I with ring. C- metaphase I with sticky. D- anaphase I with laggard. E- anaphase I with bridge. F- anaphase I with double bridges. G- telophase I with laggard. H- telophase I with broken bridge and laggard. I- telophase I with sticky .J- anaphase II with double bridges. K- disturbed telophase 2. L- telophase II with multinucleate and micronucli.

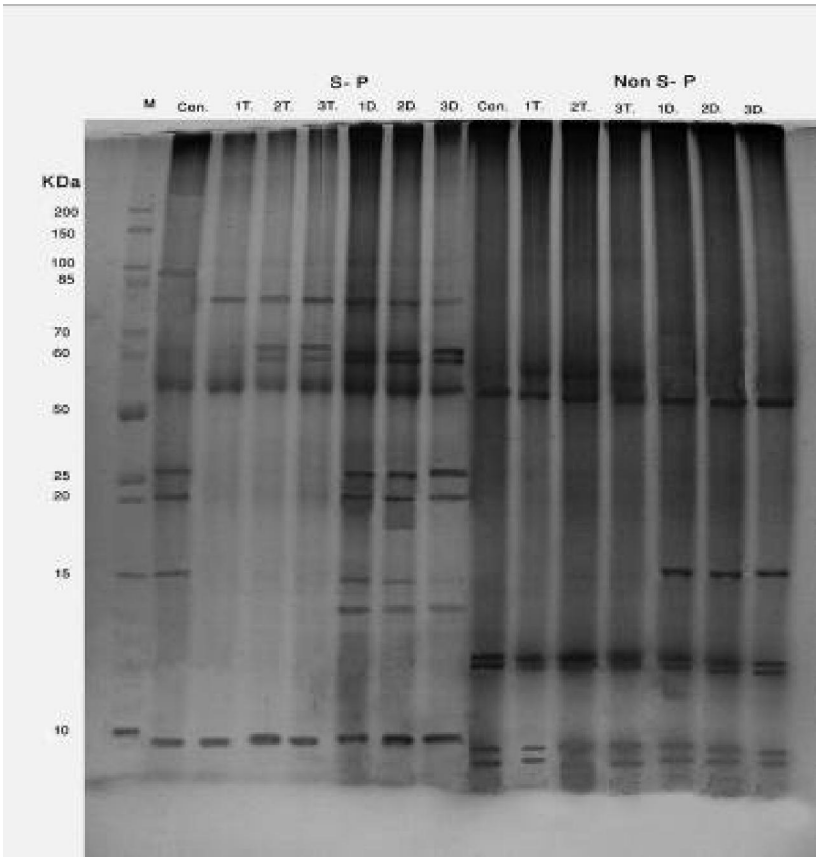

Fig (2): SDS–PAGE banding patterns of water soluble (s-p) & non-soluble (non s-p) proteins for *V. faba* after sprayed with three concentrations of Telliton (T) and Dithane (D) pesticide.

Table (3): Electrophoretic of water soluble protein banding patterns of *V. faba* seed storage protein showing the effects of three concentrations of Telliton (T) & Dithane (D) pesticides.

No. of band	MW	RF	Con.	1T	2T	3T	1D	2 D	3 D
1	102.939	0.139	+2	-	-	-	-	-	-
2	92.218	0.172	-	+2	+2	+3	+2	+2	+
3	69.238	0.258	-	-	+2	+2	+3	+3	+3
4	65.207	0.276	-	-	+2	+2	+3	+3	+3
5	56.501	0.319	+2	+2	+	+2	+3	+3	+2
6	34.965	0.463	+2	-	-	-	+2	+2	+2
7	30.196	0.507	+2	-	-	-	+2	+2	+2
8	19.320	0.641	+	-	-	-	+	+	-
9	14.171	0.734	-	-	-	-	+	+	+
10	7.325	0.932	+2	+	+2	+2	+2	+2	+2

- : Missed +: Fait +2: Dark +3: Very dark

Table (4): Electrophoretic of water non-soluble protein banding patterns of *V. faba* seed showing the effects of three concentrations of Telliton (T) & Dithane (D) pesticides.

No. of band	MW	RF	Con.	1 T	2 T	3T	1D	2D	3D
1	63.107	0.287	-	+2	+2	+2	-	-	-
2	55.478	0.327	+2	+2	+2	+2	+2	+2	+2
3	19.982	0.644	-	-	-	-	+3	+3	-
4	13.359	0.769	+2	+2	+2	+2	+2	+2	+2
5	12.485	0.790	+2	+2	+2	+2	+2	+2	+2
6	8.056	0.926	+2	+2	+	+	+	+	+
7	7.505	0.948	+2	+2	+	+	+	+	+

- : Missed + : Fait +2: Dark +3: Very dark

4. Conclusion

From the present data it may be concluded that treatment of *V. faba* plants with different concentrations of Telliton and DithaneM-45 showed positive chromotoxic effects in PMCs and changes in the M2 seed storage protein banding patterns. These effects included chromosomal abnormalities such as stickiness, laggards, bridges, disturbed, micronuclei and multinuclei. While at the biochemical level, the obtained data showed several changes in M2 seed storage protein banding pattern included alterations in band intensity, alterations in the relative mobilities of some bands, disappearance of some bands and appearance of new other bands of protein banding patterns, as compared with the negative control.

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Studies on the uptake of heavy metals by selected plant species growing on coal mine spoils in sub-tropical regions of India

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Abstract: The accumulation of heavy metals in naturally occurring plants of herbs, shrubs and trees grown on South Bolanda coal mine overburdens in subtropical region of India were illustrated.. The inter-elemental relationships of different parts of five plant species including herbs, shrubs and trees with the coal mine wastes were studied. From the tree species maximum positive correlation was observed for Cu in stem and leaf of *Trema orientalis*. The stem and leaf of *Haldina cordifolia*, *Diospyrous melanoxylon* and *Ixora arborea* showed positive correlation for Cr, Fe and Cu respectively. Among the shrubs in *Phyllanthus reticulatus*, Cr in stem showed a positive correlation with Cr in leaf. Here among five species of annual herbs, the correlation coefficient for inter elemental variable of whole plant and coal mine spoil for chromium was marked in *Catharanthus roseus*. From the above investigation it was concluded that stabilization of coal mine spoils could be achieved successfully by the plantation of suitable plant species available in native area. [Journal of American Science. 2011;7(1):26-34]. (ISSN: 1545-1003).

Key words: Coalmine spoils, Heavy metal, Inter-elemental relationship, Overburden Positive correlation.

1. Introduction

The ecology of a plant community is greatly influenced by physical and chemical properties of soil, particularly presence of excess and deficiency of mineral nutrients (Miles, 1979). Mining activity has caused serious environmental disaster besides depletion of natural vegetation and land degradation.

Establishment of a vegetation cover is essential to stabilize the bare area and to minimize the pollution problem (Das, *et al*; 1992). To remediate the adverse physical and chemical properties of the sites, the choice of appropriate vegetation will be important (Wong, 2003).

The most critical processes in the ecosystem development include colonization by appropriate selected species, accumulation of nutrients both in plants and soils, changes in soil structure and reduction in toxicity. These critical processes lead to distinct and characteristic flora (Bradshaw, 1983 and Chadwick *et al*., 1987). Harthill and Mc Kell (1979) suggested that geology- soils- plant stability circuit was disturbed by mining. Destruction of soil properties like soil productivity, soil pH, soil texture, low water holding capacity, acidity, lack of nutrients and excess of toxic metals are the major factors for the vegetative stabilization of coal mine waste (Doubleday, 1974). Metalliferous mine wastes usually contain more than one metal and these may occur at toxic concentrations (Samantaray, 1991). Coal mine wastes usually contain more than one metal and some of these might occur at toxic concentrations (Deo, 1992). The effect of single metal on plants or comparison of the toxicity of two metals have been reported in different plant species (Wong and Bradshaw, 1982). In most cases, the plants need low concentrations of minerals like Zn, Cu, Al, Cr for plant growth and metabolism and presence of these in high concentrations may indicate toxicities to plant communities. In mine waste, most of the plants grow well but some species show abnormal growth because of nutrient deficiency and presence of heavy metals (Bradshaw and Chadwick, 1980, Deo, 2005). The uptake of heavy metals by various plant species growing on chromite mine spoils were studied (Samantaray *et al* 1999). High concentrations of heavy metals depressed plant growth but certain mineral elements were required in trace for good and healthy growth (Wong and Bradshaw, 1982) . The aim of the present study is to determine the inter-elemental relationships among selected plant species growing on the overburden spoils of South Bolanda coal mine, Orissa.

2. Material and Methods

Study site

The study site South Bolanda Colliery spreads over 2582.90 acres is located within Latitude 20°54'58" and 20°55'55" and Longitude 85°07'44" and 85°11'39" in South West of NCDC'S (National Coal Development Corporation), Talcher Colliery in

20°57'-85°10' in Angul district of Orissa. The project became operational in the year 1959 and was brought under revenue account on 1.2.1961. The volume of overburden in the quarriable area has been estimated as 81.35 M.Cu.m. The area is predominantly undulating. The surface elevation varies from 103 meters in the South-East to 154 meters in the North-West above the mean sea level sloping towards south. Rock exposures are limited to few sandstone and pebble beds often stand out as small flat-topped -ridges and knolls. The climate of the area under study is subtropical with seasonal rainfall during the South-West monsoon season from June to October. Occasional rainfall breaks towards in the month of November to January. The annual rainfall ranged from 1000mm to 1500mm. The study was conducted during the three seasons of a year.

Soil sampling

Soil samples were collected from the selected coal mine overburden in different seasons of the year. The soils were collected at a depth of 0.1m by point method from the naturally occurred plants area. Specially prepared pointed bamboo pegs were used to avoid contamination and the collected samples were labelled properly. The samples were powdered by mortar and pestle in the laboratory and sieved by using a 2mm nylon sieve. The powdered samples were kept in plastic containers for elemental analysis.

Plant samples

Plant samples were collected from the coal mining overburden area where dominant number of trees, shrubs and herbs were occurred. The plants were uprooted carefully and were collected by polythene bags. Then they were brought to the laboratory washed with tap water thoroughly and then dipped in 0.1N HCl solution followed by repeated washings in distilled water. The samples were properly dried and cut into small pieces, homogenised by mortar and pestle to avoid contamination and were stored in plastic jars for elemental analysis.

Soil analysis

From the coal mine overburdens 15 samples (20g each) were collected randomly and dried at 70°C for 72 hours in the oven. One gram of dried soil from each sample was taken in a test tube. Concentrated HCl (8ml) and 2ml concentrated nitric acid in ratio of 4:1 were added and kept for over night. Diacid digestion was done on a hot plate at 105°C for 1 hour and then at 140°C until the samples were dried. After cooling, 12ml of 20% HCl by volume were added to it and the mixture was rewarmed at 80°C for 20 minutes. Then after cooling, the solution was mixed with double distilled water and homogenised by a magnetic stirrer and filtered through Whatman 42 filter paper into a 50ml volumetric flask with deionised water (McGrath and Cunliffe,1958). After filteration and dilution the digested solution was analysed for determination of Cu, Fe, Al, Cr by ICP 8410 Plasmascan (Australia) using respective wave lengths for Cu-324.754nm, Fe-238.204nm, Al-396.152nm and Cr-205.552nm.

Plant analysis

Plant samples were washed with distilled water, oven dried at 70°C in the laboratory. Powdered shoot, root and leaf samples (One gram each) of each species of tree shrub and herb (whole plant) were pre-digested in 10ml concentrated nitric acid for 12 hours followed by digestion with 5ml diacid mixture, nitric acid (HNO_3): perchloric acid ($HCLO_4$) in the ratio of 3 : 2. After that the distilled water was added to the digested samples and then filtered by Whatman-42 filter paper (Institute agronomico de St. Paulo,1978) . After suitable dilution, the samples were ready for elemental analysis by ICP 8410 Plasmascan (Australia) by using respective wave lengths mentioned before.

Statistical analysis

The data which were used on various parameters in different experiments depicted earlier were analysed statistically following statistical methods with the help of a IBM-PC computer for interpretation of the results. The significance of the correlation coefficient (r). ANOVA was used in order to establish the significance of variation between treatments at 0.05 levels. Completely Randomised Design was performed.

3. Results:

The natural vegetation of South Bolanda Coal Mine fell under the category of dry- deciduous forest type (Fig.2). The vegetation was comprised of 185 specific taxa belonging to 48 families. .Analysis of coal mine spoils samples revealed pH, 5.5; water holding capacity 11% ; organic carbon (C), 1.53% ; total nitrogen to average 0.06%; available P, 1.24 ppm; K, 125 ppm; Ca, 128 ppm and Mg , 207 ppm. The particle size of the mine spoil was sand 90%, silt 6% and clay 4%. The coal mine waste was acidic and mostly sandy loam in nature with low water holding capacity. The natural occurring plant species exhibited some morphological abnormalities in *Croton bonplandianum* with variegated leaves and clustered terminal, small sized leaves in *Catharanthus roseus.* Dwarfism and modification of floral part with induced sterility and clustering of the leaves at the top were also noted.

The inter elemental relationships of the soil alone were similar in the inter-elemental relationships for plants alone. In the present study, attempts have been made to compare the elemental composition in trees, shrubs and herbs growing at different sites (overburdens) in the mining area that were geologically and physiologically same, but differed in their mine spoil lithology and bioavailability.

The statistical data showed relationships among four heavy metals drawn with a view to study the heavy metal content of the soil and accumulation in leaf and stem of five selected tree species. From the study it was depicted that both positive and negative correlation coefficients were established between Cu, Fe, Al, and Cr in soil and in leaf and stem of *Trema orientalis*, *Haldina cordifolia*, *Diospyros melanoxylon*, *Ixora arborea* and *Tamarindus indica* (Table 1). Maximum positive corelation was observed in stem and leaf of *Trema orientalis* for copper. In *Haldina cordifolia*, chromium in stem showed relationship with chromium in leaf. In *Diospyros melanoxylon*, iron in stem and leaf had a positive relationship with a correlation coefficient study having (r = 0.886; P=0.05). In *Ixora arborea*, positive relationship was observed between chromium in stem and chromium in leaf. In *Tamarindus indica*, copper in the stem showed a positive correlation with copper in the leaf.

Here the result showed the relationship between elemental status of copper, iron, aluminium and chromium in soil and in leaf and stem accumulation of five selected shrub species. Correlation analysis revealed that there was no positive significant correlation marked between Cu, Fe, Al, and Cr and vegetative parts i.e, stem and leaf of *Chromalaena odorata, Calotropis gigantea, Woodfordia fruiticosa, Cassiaria elliptica and Phyllanthus reticulatus*. The data in the basis of bivariate correlation coefficient studies indicated that copper in stem had shown a significant positive correlation (r = 0.892; P=0.05) with copper in leaf of *Chromalaena odorata* (Table 2). Other metals, however did not show any significant relationship in other species like *Calotropis gigantea, Woodfordia fruiticosa, Cassiaria elliptica.* But in *Phyllanthus reticulatus*, chromium in stem had shown a positive correlation with chromium in leaf (r = 0.967; P = 0.05). Correlations for inter-elemental (Cu, Fe, Al, and Cr) analysis of whole plant of five selected herb species are presented in Table 3 and Table 4. The correlation coefficients analysis revealed that there was no significant correlation for copper, iron, aluminium, and chromium in whole plant of the species like Croton bonplandianum, Catharanthus roseus, Hyptis suaveolens, Solanum xanthocarpum and Tridax procumbens (Table-3).

Table 1: Correlations for inter-elemental variables between stem, soil and leaf in different tree species grown on coal mine overburden spoils of South Bolanda

Species	Element Corr. Coef. (r)	Stem vs soil Corr. Coef. (r)	Leaf vs soil Corr. Coef. (r)	Stem vs leaf Corr. Coef. (r)
Trema orientalis	Cu × Cu	– 0.210	– 0.003	0.950*
	Fe× Fe	0.349	–0.018	0.803
	Al × Al	0.046	0.466	0.167
	Cr × Cr	0.205	–0.218	–0.063
Haldina cordifolia	Cu × Cu	–0.607	–0.658	0.636
	Fe× Fe	–0.558	0.752	–0.016
	Al × Al	0.275	0.529	0.828
	Cr × Cr	–0.712	– 0.598	0.923*
Diospyros melanoxylon	Cu × Cu	0.470	–0.305	0.251
	Fe× Fe	–0.032	–0.473	0.886*
	Al × Al	0.328	–0.085	0.805
	Cr × Cr	–0.289	0.629	–0.224
Ixora arborea	Cu × Cu	–0.187	0.695	0.460
	Fe× Fe	0.789	0.268	0.209
	Al × Al	–0.445	0.042	0.809
	Cr × Cr	–0.114	–0.383	0.952*
Tamarindus indica	Cu × Cu	–0.640	–0.486	0.911*
	Fe× Fe	–0.693	–0.072	0.629
	Al × Al	–0.476	0.119	0.686
	Cr × Cr	0.078	0.643	0.750

Table 2. Correlation for inter elemental variables between soil, stem and leaf in different shrubs grown on coal mine overburden spoils of South Bolanda

Species	Element Corr. Coef. (r)	Stem vs soil Corr. Coef. (r)	Leaf vs soil Corr. Coef. (r)	Stem vs leaf Corr. Coef. (r)
Chromalaena odorata	Cu × Cu Fe× Fe Al × Al Cr × Cr	0.575 0.010 –0.480 0.225	0.244 0.213 0.697 –0.647	0.892* 0.433 0.784 0.330
Calotropis gigantea	Cu × Cu Fe× Fe Al × Al Cr × Cr	–0.947 –0.453 0.139 0.075	0.066 –0.898 0.625 – 0.421	0.015 0.631 0.087 –0.934
Woodfordia fruiticosa	Cu × Cu Fe× Fe Al × Al Cr × Cr	0.299 0.393 – 0.334 0.100	– 0.379 0.421 – 0.334 0.603	–0.160 0.827 0.093 0.228
Cassiaria elliptica	Cu × Cu Fe× Fe Al × Al Cr × Cr	–0.453 –0.446 –0.359 0.487	–0.299 – 0.875 0.512 0.019	0.703 0.656 0.348 0.726
Phyllanthus reticulatus	Cu × Cu Fe× Fe Al × Al Cr × Cr	0.549 –0.173 0.387 0.604	–0.188 –0.387 –0.623 0.479	0.206 0.624 –0.068 0.967*

Table 3.Correlation for inter-elemental variables between whole plants (herbs) grown on the coal mine overburden spoils of South Bolanda

Species	Element Corr. Coef. (r)	W. P vs W.P Corr. Coef. (r)	
Croton bonplandianum	Cu × Fe Cu × Al Cu × Cr Fe × Al Fe × Cr Al × Cr	–0.626 –0.609 –0.046 –0.335 0.732	–0.601
Catharanthus roseus	Cu × Fe Cu × Al Cu × Cr Fe × Al Fe × Cr Al × Cr	– 0.429 –0.283 0.217 0.533 –0.671	0.224
Hyptis suaveolens	Cu × Fe Cu × Al Cu × Cr Fe × Al Fe × Cr Al × Cr	– 0.215 0.296 0.576 0.856 0.104 0.373	
Solanum xanthocarpum	Cu × Fe Cu × Al Cu × Cr Fe × Al Fe × Cr	0.007 0.249 0.533 0.774 –0.323	

	Al × Cr	–0.070
Tridax procumbens	Cu × Fe Cu × Al Cu × Cr Fe × Al Fe × Cr Al × Cr	–0.871 0.146 0.003 –0.168 –0.317 0.321

Table 4.Correlation for inter-elemental variables between whole plants (herbs) and soil grown on coal mine overburden spoils of South Bolanda

Species	**Element Corr. Coef. (r)**	**W. P vs soil Corr. Coef. (r)**
Croton bonplandianum	Cu × Cu Fe × Fe Al × Al Cr × Cr	–0.248 0.281 0.019 –0.344
Catharanthus roseus	Cu × Cu Fe × Fe Al × Al Cr × Cr	–0.401 –0.729 –0.159 0.833
Hyptis suaveolens	Cu × Cu Fe × Fe Al × Al Cr × Cr	0.364 –0.432 –0.667 0.047
Solanum xanthocarpum	Cu × Cu Fe × Fe Al × Al Cr × Cr	–0.247 0.615 0.103 0.339
Tridax procumbens	Cu × Cu Fe × Fe Al × Al Cr × Cr	0.794 0.065 0.537 0.273

Fig.1: Coalmine overburden of South Bolanda

Fig.2: Coalmine overburden with sparse vegetation (South Bolanda)

4. Discussion:

The overburden exhibited a poor vegetational cover with greater dominance of herbaceous members. The vegetation of old dumpsites were comparatively richer with the establishment of large number of shrubs and arboreous members with stunted growth .Similar type of vegetation distribution was observed in different coal mine sites and reported by several workers (Wali and Freeman1973, Glenn-Lewin, 1979, Jha and Singh, 1990). High amount of Fe, Mn, Ni, Mg, Al and Sulphate and poor supply of Ca, K, P and N resulted in sparse plant strands and retarded plant growth (Barnhisel and Massey, 1969). The coal mine wastes was generally acidic in nature having low water holding capacity was also noted in many coal mine spoils (Mays and Bengston, 1978, Pederson *et al*., 1980). Distribution, growth and sparse occurrence of plant species on coal mine spoil due to low nutrient and low water holding capacity was reported earlier (Deo, 1992). The natural occurring plant species exhibited some morphological abnormalities in some plant species due to sandy soil nature, low nutrient availability, presence of heavy metals and bituminous coal products of the South Bolanda coal mine spoils (Sahu *et al.*, 1989).

Data on elemental composition in the plant species growing at different mine sites showed variation (Erdman and Gough, 1979, Gough and Severson,1989). The inter elemental relationships of the soil alone were similar in the inter-elemental relationships for plants alone.

Comparison of concentrations of different elements in plant and soil showed that some of the trees, shrubs and herbs could be useful in geochemical prospecting .The statistical data showed relationships among four heavy metals drawn with a view to study the heavy metal content of the soil and accumulation in leaf and stem of five selected tree species. Various interactions of heavy metals between plant and soil were studied. (James and Bartlett, 1984) . The same trend was observed by (Gartside and McNeilly, 1974) studied plants differed remarkably in their metal contents on metal enriched soils. Accumulation of heavy metals by higher plants in metalliferous soils was also studied (Maywald and Weigel, 1997). Transport and accumulation of heavy metals in different plant species have been reported earlier in other mine spoils(Haritonidis and Malea,1995).

Elemental composition on the plant species growing at different mine sites showed variation (Munshower and Newman,1980; Erdman and Gough, 1979; Gough and Severson,1981). Here the result showed the relationship between elemental status of copper, iron, aluminium and chromium in soil and in leaf and stem accumulation of five selected shrub species. Similar trend of observation was reported (Lyon *et al.*, 1968).The distribution of heavy metals in different plant parts were reported earlier (Bower and Melhuish, 1988). Uptake of heavy metals by various plant species (trees, shrubs and herbs) in chromite spoils were studied (Samantaray *et al.,* 1999) . From the correlation analysis it was concluded that there was no significant relationship in uptake of heavy metals from the soil to the vegetative parts of the

plant. It might be due to the limited supply of heavy metals from soil to plant (Newman *et al* , 1985).The physiological mechanism of the effects of heavy metals on different plant species is still not clear, though there is evidence that most metals produce a similar kind of metabolic disturbance. A leaf necrosis appears which is specific to a particular metal, but there is also a general chlorosis of the younger leaves common to all metals (Bradshaw *et al* 1978*)*

From the five numbers of herb plant species the maximum correlation coefficient was observed in whole plant and soil for chromium in Catharanthus roseus (r=0.833; P=0.05) (Table 4). The distribution of heavy metals in different parts of plant species were reported (Lyon et al., 1968, Samantaray et al., 1999).

Thus among the various sample studied, there could be a range of genetically different plant species comprising of trees, shrubs and herbs which adapted to their own level of soil metal status or with a specific elemental uptake. It would appear that genetic variation was a minor factor where high concentration of an element was found. Cu and Fe were essential elements, and could have favourable growth; where as Al and Cr might be some concerned. From this study it was concluded that there was no correlation coefficient among the mine spoil and natural occurring plants. Overall the coal mine overburden soil is non toxic to plants. The success of reclamation schemes is greatly dependent upon the choice of species and their method of establishment. Many factors have to be considered in the choice of plant materials in particular the nature of the soil, the prevailing climate and the choice of eventual land use. So it is recommended that the plantation of suitable trees and shrubs could be come out successfully in the barren overburden of the coal mining area. The long term effects of the accumulation from the soil and their uptake by plants need further study.

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Credit and money market of the bank of the central Africa States (BEAC)

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Abstract: In a context of world economic crisis, our article on the credit and money market aim to emphasize the influence of the bank of the States of Africa on the saving in each one of its members in general and on Chad in particular through the service of credit and money market. [Journal of American Science. 2011;7(1):35-39]. (ISSN: 1545-1003).

Key words: BEAC, Credit, Money Market, Interbank market, obligatory reserves

1. Introduction

The bank of the central Africa States (BEAC), is a multinational financial institution creates by a monetary cooperation agreement of November 22nd, 1972 with Brazzaville between 5 countries of central Africa (Chad, Gabon, Congo, R.C.A. Cameroon) and France. Another country, Equatorial Guinea joined this unit on January 1st, 1985. France is member of convention without being in the authorized capital which rises to 88 Billion FCFA distributed in a leveling way between the Member States.

The BEAC is charged to emit on a purely exclusive basis the currency on the territory of the States members and to guarantee stability of it. It is also charged to lead the policy of exchange and the management of monetary reserves (through an account open to the French treasure).

The currency in force in the 6 African countries is CFA franc (FCFA) or franc of the financial co-operation in Africa. The convertibility of this currency is guaranteed by France and the backing of the FCFA to the French franc was deferred on the Euro at the time of the advent of this currency common to several European countries.

Nowadays, countries having the BEAC as issuing house belong to the Monetary Union of central Africa, which in its turn is integrated in the Economic community and Monetarist of Africa Central (CEMAC).

2. Framework of the BEAC.

Yaoundé is the head office of the BEAC. It is directed by a Governor (Gabonese) assisted of a Vice-governor (Congolese) and a General Secretary (Chadian). With these three personalities which until 2007 constituted the Government of the bank are added, in 2008, three Managing directors of nationalities Cameroonian, Equato-Guinean and Central African. Since July 2008 the government consists of 6 leaders. The government of the central bank is based on 12 operational central managements decisional framework of the seat thus counts 18 personalities named by the governments of the States members.

On the level of each State, the BEAC is represented in each capital by a National management directed by a National director who is appointed by the Board of trustees, on a proposal from the governor and after approval of the local government. Centers are set up through each country according to the level of economic activity. In France, the bank is represented by a delegate of the Governor named by this last among the senior officers.

In Chad, the BEAC is presented inside the country in the towns of Moundou and Sarh. A deposit of tickets and currencies are placed under the management of the departmental treasure of Abéché, city where it is envisaged to build an office of the central bank. National management currently counts 12 services of which that of the credit and money market.

3. Money market and service of the credit

The service of the credit and money market (CPM) is the basic operational structure of the BEAC changed to apply the decisions of monetary policy made by the monetary policy committee.

The CPM was created in November 2007 at the time of reforms institutions of the CEMAC. It is governed by articles 38 to 45 of the statutes of the BEAC and its principal roles concern:

-The definition of the monetary policy,

-The fixing of the Rates D intervention of the BEAC and the rates of taking away and remuneration of the obligatory reserves,

-The definition of the orientations as regards management of monetary reserves

The CPM consists of 14 members because of 2 members per State and 2 for France. It is chaired by the governor of the Central bank.

The fundamental objective of the money market east to slow down the exit of the capital, while seeking to retain the financial resources on the ground of the

States members of the BEAC and to direct them towards the financing of the producing and profitable operations, so as to support economic development.

4. Flow chart of the Service Credit and Money market.

The service is capped by a person in charge named by the National director and who ensures the supervision and the coordination of the activities which are reserved for him. The departmental manager has under his orders three agents: an Assistant of Direction in charge of the section Money market and Capital, a countable Assistant in charge of the Section Credit and a Private Secretary.

5. Activities of the service credit and money market

The service Credit and money market manage three shutters of the monetary policy: the credit; the money market and obligatory reserves.

5.1Credit

The BEAC can be caused to lend money in the short or medium term to the primary banks to enable them to conclude their activities of financing of the economy. This contest of the central bank is called the refinancing.

It often arrives that a primary bank receives from a customer a request for financing of a very important amount. If it does not have enough resources to satisfy this request immediately, it can in this case be turned over towards the central bank to borrow the requested amount and to lend it to the customer. But the primary bank must assemble a complete record on its petitioning customer, according to a preset groundwork by the central bank, it is known as allowed << in agreement of classification >> or << individual authorization of mobilization >>. The central bank pours all to him or part of the amount desired by the customer, condition of giving as a preliminary in guarantee of the commercial drafts subscribed by the aforementioned customer.

The files admitted in agreement of classification can be of short or medium term. The agreements of medium-term classification relate to only the open in the medium term revocable appropriations in favor of the nationals for the refinancing of the real investments. The in the medium term revocable appropriations, granted for one duration ranging between 2 years and 10 years, are refinanced by the issuing house to a total value of 80%, the remainder representing the personal capital contribution of the borrower. The effects subscribed on these appropriations and deposited in guarantee are mobilizable to 90% of their nominal.

Generally, the banks always may find it beneficial to constitute at the BEAC a mattress of dimensioned signatures for which they can constantly lodge requests for agreement of classification to obtain liquidities titrates recall, all the branches of industry, except for the research departments, are eligible with the agreement of classification.

The refinancing on agreements basis of classification is done only through level 2 of the money market.

Beside the agreements of classification, it is the agreements known as of mobilization which relate to only the refinancing of the medium-term appropriations (7 years maximum) requested on signature of the companies engaged in operations of productive investments and thus creators of wealth and employment. The eligible files for this reason are refinanced with the counter B and the agreements of mobilization given by the BEAC are irrevocable.

5.2. the money market.

The money market on two levels. Level 1 is called the interbank market and the second level relates to the interventions of the central bank.

5.2.1. The interbank market

It is the level of the money market where the banks exchange between the unconditional liquidities of amounts, rate, of duration and freely discussed guarantees. The BEAC does not intervene in these negotiations between primary banks. These last however have the obligation to inform the institute resignation of the characteristics of the transactions made within this framework.

It is necessary however to relativize the freedom which exists on the interbank market because if the interbank rates are too high or too low, the central bank will intervene directly or indirectly, either while injecting or by puncturing liquidities, or while lowering or by raising its rate which is the rate interest of invitations to tender (TIAO), in order to support the economic activity.

5.2.2. The interventions of the central bank.

According to its monetary policy, the BEAC can intervene to inject or withdraw the liquidities in the banking system. The injection and the puncture of liquidities obey very precise rules and borrow two channels:

1) The counter A or principal channel which makes it possible the central bank to control the liquidity of the trade banks by injecting the money complement necessary or by withdrawing (while puncturing) liquidities when there is too much in the banking system. The injection of liquidities is an advance made by the central bank, while the puncture is a placement carried out by the primary banks. These two operations are done by positive invitations to tender (injections of liquidities with 7jrs) and negative (punctures of liquidities to 7.28 or 84 maximum days).

2). The counter B or special channel is intended only for the granting of the loans and advanced in the medium term irrevocable at the primary banks for the refinancing of the productive investments. This counter is, by construction, offered of liquidities.

The characteristics of the counter B have the following ones:

-NR are allowed there that the requests for refinancing presented by the trade banks for the companies engaged in productive investments of wealth and employment

-When the BEAC gives its agreement of refinancing under the counter B on a file, this agreement is called individual authorization of medium-term mobilization

-The agreement of the central bank is irrevocable and gives place to the perception of the commissions of waiting and of engagement calculated on incur credit. An agreement of medium-term mobilization becomes null and void if the credit did not have a beginning of use after one year (or two years acts of the operations of production or heavy equipment).

-The refinancing of the BEAC is reached a maximum to 60% of the capital cost and the effects representative of these appropriations are mobilizable to 100% of their nominal.

The duration of in the medium term irrevocable loans lies between 2 years minimum and 7 years maximum

-The rate initial interest of in the medium term irrevocable appropriations is TIAO.it can be revised with the fall semi-annually if the last six months the balanced average TIAO is lower than the initial rate. It can also be re-examined with the rise under the same conditions without being able to exceed the initial rate.

The operations of counter A are initiated by the BEAC. But a commercial bank can be also addressed of its liking at the central bank to require a contest. In this case, four types of facilities can be to him granted:

-The catch in pension, with a rate D interest higher than that of the invitations to tender. Its duration varies from 2 to 7 days and the commercial bank must subscribe and give in guarantee a total ticket of mobilization.

-Exceptional advance guaranteed by the certificates of placement, with the interest rate of invitations to tender (TIAO) and over one duration not exceeding that of the validity of the certificates.

T-he advance intra-day laborer: granted for one day to a bank in rupture of liquidity on its account to the BEAC and guarantee by a total ticket of mobilization .l' specific intervention is an exceptional facility which is granted only in the event of going beyond the objective of refinancing (maximum quantity of currency, evaluated during the monetary programming, that the central bank can put at the disposal of L saving in a country)

All the advances of the central bank are guaranteed by commercial drafts or pledges - species (certificates of placement).

5.3. The obligatory reserves

The obligatory reserves consist of part of the deposits of the trade banks that the central bank retains authority in its trunks. It is an additional tool of regulation of the liquidity of banks .II acts to prevent the primary banks from having too much cash which they would be tempted to place in a ill-considered way through easy loans at the private individuals and the companies. It is known that only a rare good has value. The tight money thus should be made so that it keeps its value and that he does not cause inflation by his abundance.

The decision to subject the banks to the constitution of obligatory reserves goes back to 1999 and it came into force on August 23rd, 2001.

The decision to subject the banks to the constriction of obligatory reserves goes back to 1999 and it came into force on August 23rd, 2001.

Reserves are carried out monthly on the whole

of the deposits of each bank, namely the sight deposits and the term deposits. Currently, for the banks of Chad, the sight deposits are retained with height of 7.75% and those in the long term to 5.25% (decision CPM March 2009). It should be noted that the variation of the proposals for a taking away between the two types of deposits is explained by the fact why the first are increasingly more important in the banks.

Besides their differentiation by type of deposit, the minimum reserve ratios are also differentiated by groups from country constitute according to the similarity of the liquidity of their economies. Thus, at March 23rd, 2009, the monetary policy committee set up four groups of State according to their economic situation and the coefficients of reserves brackets at the banks which are there establish arise as follows:

Table 1, economic situation and coefficients of reserves brackets

	Group I	Group II	Group III	Group IV
Criteria	Location ample liquidity	Location satifactory liquidity	Location sufficient liquidity	Location insufficient liquidity
Countries	Congo Equatorial Guinea	Cameroon	Chad	RCA

		Gabon		
Coefficients of deposits	14,0%	11,75 %	7,75 %	0 %
Coefficients for time deposits	10,50 %	9,25 %	5,25 %	0 %

Exceptionally, and taking into account the socio-economic situation of RCA, the banks in activity in this country are exempted taking away of the obligatory reserves.

The obligatory reserves are remunerated. The rate credit interest is of 0.10% (decision of March 23rd, 2009).

Only the banks in serious cash shortage or situation of reorganization or liquidation can be exempted constitution of obligatory reserves.

6. The advances counts some running to the Treasury.

The BEAC is also the bank of the Chadian State but this one cannot draw the money there as he wants. Indeed, as he wants. Indeed, as it is known as higher, the BEAC belongs to six countries and this characteristic implies very rigorous rules of management to guarantee the value of the currency.

Each State can secure loans from BEAC.She are reached a maximum to 20% of the annual budget revenue ordinary of national

origin, which excludes the receipts coming from the gifts, of the subsidies, loans and of the former exercises. The threshold of 20% constitutes the ceiling of maximum advances that the Treasury can obtain.

At the time of the determination of the ceiling of advances in the State, if it is that government stock was mobilized with the counter of the institute resignations by the primary banks on the signature of this State; the amount of this mobilization is deduced from the new ceiling.

The advance is granted for one twelve months maximum duration. However, it can happen that State tests difficulties of refunding

the advances and this situation can lead to their consolidation in a medium-term loan.

In the near future, the recourse in advance of the Central Bank, whom one calls also monetary financing of the State, will disappear. Indeed, to finance their operation and their investments, the States of the CEMAC decided to resort from now on to the emission of the public titles for raising funds on the market. The project concerning with this way of financing was officially launched in 2008 and will have to come into force on July 1st, 2009.

7. Formation of the interest rates.

Apart from the rates of the interbank compartment of the money market which are freely discussed by the trade banks, the other rates applied by the BEAC are fixed by the CPM. The relating to it decisions are based on criteria such as the level of the economic activity, the tendency of the rates external to zone CEMAC, the level of the liquidities of the banking system, the position of the account of operations near the French treasure, etc

The grid of the rates in force is the following one after the decisions taken by the CPM the 6/29/2009:

Table 2, The grid of the rates in force

Rates	AIMED OPERATION	Observations
	Debtor rates	
Interest rate of the invitations to offer: TIAO	Injection of liquidities in the banking system	It is the directing Rate of the BEAC : 4.50%
Rate interest of the catches in pensions: TIPP	Injection of liquidities in the banking system	It is equal to the raised TIAO from 1.5 to 3 points (lasted from 2 to 7 days): 6.25% at March 23rd, 2009
rate of penalty at the banks: TPB	Sanction and injection of liquidities in the absence of eligible effect at the money market	12% at March 23rd, 2009
Rate for advances to the treasure inside the statutory ceilings	Monetary financing of the State	4.5% at March 23rd, 2009
Rate for advances to the treasure	Monetary financing of the State	10% at March 23rd, 2009

Table 3, the grid of the rates in force

Rate	AIMED OPERATION	OBSERVATIONS
Beyond the statutory ceilings	State	
Maximum debtor rate	Limitation of wear. Any rate higher than the TDM is liable to pursuit	It is equal to the TPB raised of a margin of 7% fixed by CA of the BEAC on November 24th, 1995. Since July 3rd, 2008. This rate was removed (Decision CP M of July 2nd, 2008)
CREDITORS RATE	TAUX CREDITEURS	CREDITOR RATE
Interest rate on the placements at 7 days: TISP	Puncture of liquidities	1% at March 23rd, 2009
Interest rate on the placements at 28 days : TISP	Puncture of liquidities	1 % raised of 1 /16 point
Interest rate on the placements at 84 days : TISP	Puncture of liquidities	1 % raised of 2 /16 point
Rate of remuneration of the obligatory reserves	Puncture of liquidities	0,10 % at March 23rd, 2009
Interest rate on the public placement under the reserve funds for the future generations : TISPP 0	Placement of the surpluses of the oil States	1,9 % at March 23rd, 2009
Interest rate on the public placement under the mechanism of stabilization of the budget revenue : TISPP 1	Placement of the surpluses of the oil States	1,7 % at March 23rd, 2009
Interest rate on public placement under the special deposits : TISPP2	Placement of the surpluses of the oil States	1,1 % at March 23rd, 2009
Minimum creditor rate : TC M	Encouragement with the public saving, with a ceiling of deposit on booklet has 5 million FCFA	Since July 3rd, 2008, TC M is 3.25% (Decision of the CPM of July 2nd, 2008)

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Women's Empowerment for Rural Development

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Abstract: The main objective of this study provides a strategy for women's empowerment for rural development. Empowerment can enable women to participate, as equal citizens, in the economic, political and social sustainable development of the rural communities. The findings outlined in this paper suggest that, designed and implemented in ways that meet rural women's diverse needs, community participation processes that can be important to facilitating social, technological, political and psychological empowerment in terms of rural development. The findings of this investigation can assist rural developers in the implementation of community development strategies based on women's empowerment.

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Keywords: women's empowerment, rural development, local development

Introduction

Since the 1990's women have been identified as key agents of sustainable community development and women's equality and empowerment are seen as central to a more holistic approach towards establishing new patterns and processes of development that are sustainable (Handy & Kassam, 2004). The World Bank has suggested that empowerment of women should be a key aspect of all social development programs(Bank, 2001). The term 'empowerment' is a contested concept which connotes different meanings depending on different perspectives of looking at it (Asnarulkhadi & Aref, 2009). The empowerment of women means for them to have the necessary ability to undertake a number of tasks either individually or in groups, so that they have further access to and control of society resources. Empowerment is recognized as an essential strategy to strengthen the well-being of individuals, families and communities, government and non government agencies (Aref, 2010). In other word empowerment is an abiding process which takes place with specific intent so enabling them to have further control over society's resources. (Rezaei, 2007). Numerous studies of empowerment have been published (Aref & Ma'rof, 2009; Aref et al., 2009; Gillman, 1996; Gore, 1992; Humphries, 1994; Lennie, 2002; Peters & Marshall, 1991). This literature suggests that rural researchers need to adopt a more critical approach to the concept and to be more explicit about the processes they claim have facilitated empowerment. This requires the development of useful models of empowerment and effective methods for evaluating and critically assessing claims for empowerment (Anderson, 1996).

A model of rural women's empowerment

Drawing on Friedmann's framework (Friedmann, 1992) and the meanings and indicators of empowerment identified in the analysis, Figure 1 presents the model of rural women's empowerment that was developed (Lennie, 2002). This illustrates the interrelationships between the four forms of empowerment that were identified, and summarizes the key features of each form of empowerment. Although these four forms of empowerment are discussed separately in this paper, there are clearly many interrelationships and overlaps between them (Lennie, 2002).

The types of women' empowerment

The major types of empowerment can be summarized into four groups (Lennie, 2002).

Community empowerment: Access to new and useful knowledge and awareness, Developing new skills, abilities, confidence and competence, obtaining the friendship and support of other women, participating in various activities with other women.

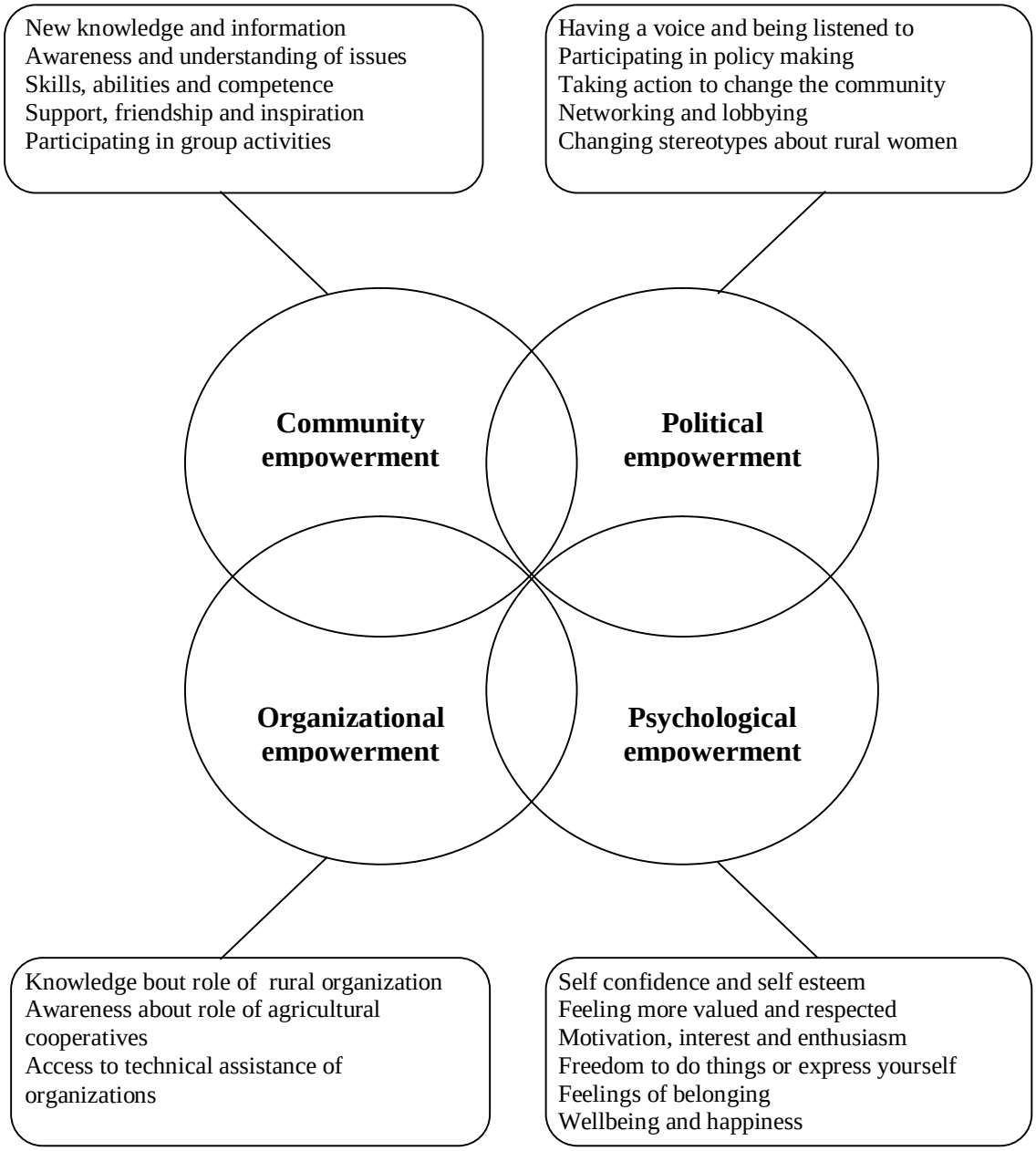
Organizational empowerment: New knowledge and awareness about new benefits of technology for rural development through rural

tourism development or development of agriculture cooperatives.

Political empowerment: Influencing other government policies and decisions that affect on rural communities, changing town-based people's beliefs, networking with people in government and industry and other women to discuss issues affecting rural women and rural communities.

Psychological empowerment: An increase in self-confidence and self-esteem, Greater motivation, inspiration, enthusiasm and interest to develop new skills and knowledge, to keep pushing for better services for rural people, feelings of belonging related to participation in the online groups in particular (Lennie, 2002).

Figure 1: The key forms and features of rural women's empowerment Adapted from (Lennie, 2002)



Conclusion

The result of this study is suitable for the empowerment of rural women for take control of the management of local development in their villages. Because with the empowerment of women, the elimination of gender discrimination and the creation of a balance of power between men and women, will not only be beneficial to women, but society as a whole shall benefit politically, economically and culturally. The results of this review suggested a range of strategies that could enhance rural women's empowerment, including the use of agricultural cooperatives in this process. Organizational empowerment through agricultural cooperatives was identified as a significant approach to achieving the rural development.

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Inhibitory effects of two indigenous plant extracts (*Zingiber officinale* and *Ocimum gratissimum*) on post harvest yam (*Dioscorea rotundata Poir*) rot, *in vitro*.

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Abstract: Cold water and ethanol extracts of two fungicidal plants (*Zingiber officinale* and *Ocimum gratissimum*) were screened for their *in vitro* effects on rot fungi of yam using 60 and 80% aqueous extract and 20 and 30% ethanol extract of each concentration. The two concentrations of aqueous and ethanol extracts were found to have inhibitory effects on all the rot fungi isolated from yam, 80% *a*queous extract of *Zingiber officinale* inhibited *Fusarium oxysporum* to 66.70%, 80% aqueous extract of *Ocimum. gratissimum* inhibited *Botrydioploidia theobromae* to 60.00% also73.33% inhibition of *Aspergillus flavus* was recorded using 30% ethanol extract of *Zingiber officinale*, the same concentration of *Ocimum gratissimum* inhibited *Aspergillus niger* to 70.00%. Both aqueous and ethanol extract of *Zingiber officinale* and *Ocimum gratissimum* had potential inhibitory effect on all the rot fungi.

[Ijato James Yeni. **Inhibitory effects of two indigenous plant extracts (*Zingiber officinale* and *Ocimum gratissimum*) on post harvest yam (*Dioscorea rotundata Poir*) rot, *in vitro***. Journal of American Science 2011;7(1):43-47]. (ISSN: 1545-1003). http://www.americanscience.org.

Key word – *In vitro*, *Zingiber officinale*, *Ocimum gratissimum*, rot fungi, yam.

Introduction

Several fungi have been identified by different workers as causal organisms of many plant diseases. Adebanjo and Onesirosan (1986) isolated *Colletotrichum gloesporoides* as a fungal pathogen infecting minisetts through infected yam tubers. Ikotun (1989) also isolated many fungi associated with rot of yam tubers.

Synthetic fungicides control approach has proved effective in the control of phyto diseases. Yam has been protected against rot using borax (5% aqueous solution), copper 8 – hydroxyquinolinoate (4% aqueous solution) and lime ash (Coursey, 1961). Ogundana and Denis (1981); Plumbey (1985) enumerated many other synthetic fungicides active against some rot causing phyto pathogens. Nwakiti *et al.* (1990) listed some defensive synthetic fungicides which have been proved efficacious in controlling some phyto pathogens; they averred that those fungicides were costly and therefore are not economically viable. The continuous and unguided use of chemicals in agricultural processes potent an acute and chronic toxicity to man and livestock (Kamel and Manga, 1987).

The use of these chemicals can result to death by accumulating in man, poison and concentrate in the food chain as they are usually not eco – friendly. They can induce resistance and resurgence of phyto pathogens, pest population and as well as cause death of flower pollinators, predators and parasites. In this respect, it is necessary to search for dependable and sustainable antidotes that regard the requirement of man and its environment.

The use of pesticides of botanical origin has been pin – pointed by many researchers as an option to synthetic fungicides, as an alternative to the havoc and contamination difficulty identified with the indiscriminate application of fungicides (Amadioha, 1998, 2000; Amadioha and Obi, 1998, 1999). Therefore, the effectiveness of botanical extracts requires investigations. Ejechi and Ilondu (1999) used the sawdust extract from cam wood to control yam rot caused by *Sclerotium rolfsii*. The significance of natural bio pesticides as possible sources of pathotoxicity as they are systematic and easily biodegradable has been stressed in the separate work of (Al Abed *et al* 1993, Amadioha and Obi 1998, 1999, Amadioha 2000 and Olufolaji, 1999; Okigbo, 2009).

This investigation is therefore targeted at the inhibitory effects of two tropical, indigenous plant extracts of *Zinginber officinale* and *Ocimum gratissimum* on yam rot fungi at *in vitro* study.

Methodology

Collection of yam tubers that showed symptoms of softness were collected from the market at Ado-Ekiti, Nigeria. Yam tubers with softness of tissues were identified as being rotted, fresh and healthy yam tubers were also collected, packed into a polythene bag already lined with tissue paper and taken to the laboratory for further studies.

Collection of plant materials

Ocimum gratissimum was purchased from the market at Ado- Ekiti while *Nicotiana tobacum* was collected in the vegetation reserve of Ado-Ekiti, Nigeria. These plants were taken to the herbarium unit of University of Ado-Ekiti for proper identification.

Isolation of spoilage fungi from rotten yam tubers.

Pieces of yam tuber 3 x 3 x 2mm in dimension was cut from advancing edge of a rot, they were surface sterilized in 70% alcohol for 1minute and dried on sterile tissue paper and plated out on potato dextrose agar (PDA) already incorporated with streptomycin. A minimum of three replicated pieces from each of the rotted portion were plated out. The plates were incubated at room temperature for five days and fungi associated with rot affected tissue were identified and their frequency of occurrence determined using method of Okigbo and Ikediugwu (2000).

Pathogenicity tests

The method of Okigbo and Ikediugwu (2000) was used, cylindrical cores, 1cm deep, were removed from various spots of a healthy yam tuber with sterile 5mm cork borer and then 4mm discs taken from the edge of a colony of test fungus were place downward into each of the holes in the tuber. The cores of the yam tuber were replaced after 2 mm pieces has been cut off to compensate for the thickness of the agar inoculums and the replaced core sealed with melted candle wax. Sterilized PDA was used in place of the culture disc served as control.

Preparation of plant extracts.

Zinginber officinale and *Ocimum gratissimum* were air dried and grounded separately. Thirty grams of each sample was added to 15ml of distilled water in separate flasks. This was vigorously stirred and last to stand for 24 hrs. The sample was filtered with a Whatman filter paper (No.1) and the filtrate used as the extract. Same process was followed using 30 and 20% ethanol extract concentrations.

Effect of plant extracts on fungal mycelia growth

The method of Amadioha and Obi (1999) was used to determine the effect of the extract on fungal growth. This involved creating a four equal section on each Petri-dish by drawing two perpendicular lines at the bottom of the plate, the point of intersection indicating the centre of the plate. This was done before dispensing PDA into each of the plates. About 2ml of the extract of various plant materials were separately introduced into the Petri-dish containing the media (PDA). A disc (4mm diameter) of the pure culture of each isolate was placed on the extract just at the point of intersection of the two lines draw at the bottom of the Petri dish. Control experiments were set up without addition of any plant material. Fungitoxicity was recorded in terms of percentage colony inhibition and calculated according the formula:

$$\text{Growth inhibition (\%)} = \frac{DC - DT}{DC} \times \frac{100}{1}$$

Where: DC -Average Diameter of control and

DT -Average diameter of fungal colony with treatment.

Result

The isolated fungi from rot affected yam included *Fusarium oxysporum, Fusarium solani, Aspergillus flavus, Aspergillus niger, Botryodiplodia theobromae* and *Rhizopus stolonifer*. These fungi were found to cause yam rot from the pathogenicity test. Rot was determined by the softness of tissues. Extract of both *Zingiber officinale* and *Ocimum gratissimum* (cold aqueous and ethanol extract) were found to possess fungitoxic effect on the radial growth of the rot fungal mycelia (Tables1 and 2). There was no remarkable difference in the concentrations of aqueous extract of *Z. officinale* as well as *Ocimum gratisssium*. The highest inhibition of 60.00% was recorded on 60% aqueous extract of *Z. officinale* against *B. theobromae*, whereas 80% aqueous extract of the same test plant inhibited *F. oxysporum* to 66.70%. The highest inhibition using 80% aqueous of *Ocimum gratissimum* was on *B. theobromae* of 60.00% (Table 1). 70% inhibition was observed with 30% ethanol extract of *Z. officinale* on *F.*

oxysporum, 66.66% inhibition was recorded on *B. theobromae* (Table 2) 70% inhibition was obtained using 30% ethanol extract of O. gratissium on *A. niger* being the highest followed in order by *B. theobramae* and *A. flavus* of 66.66% inhibition (Table 2). *B. theobromae* appeared to be the most inhibited by both aqueous and ethanol extracts of the test plants. The highest inhibitory of ethanolic extract was found using 20% ethanol of *O. gratissimum* on *A. flavus* of as much as 73.33% (Table 2).

Table1**.** Percentage inhibition of mycelia radial growth of rot fungi in Potato dextrose agar with aqueous plant extracts of 60% and 80% concentrations. Plant extracts (% inhibition of mycelia growth).

Rot fungi	*Zinginber Officinale*		*Ocimum gratissimum*		Control (mm)
	60%	80%	60%	80%	
A.niger	46.66cd	56.66cd	40.00bc	53.33bc	30.00
F.oxysporum	46.66cd	66.70a	46.66a	46.66d	30.00
R. stolonifer	41.17e	52.94bc	35.30c	46.66d	34.00
B.theobramae	60.00a	46.66d	40.00bc	60.00a	30.00
A. flavus	45.16de	51.62cd	45.16ab	51.62cd	31.00
F. solani	50.00bc	53.33b	50.00a	53.33bc	30.00

Each of the data is a mean of three replacements. Each data followed by the same alphabet along the columns not significantly different at P= 0.05 using (DMRT) Duncan Multiple Test to separate the means.

Table 2**.** Percentage inhibition of mycelia radial growth of rot fungi in Potato dextrose agar with plant ethanol extracts of 20 and 30% concentrations. Percentage inhibition of mycelia growth (means)

Rot Fungi	*Zingiber officinale*		*Ocimum gratissimum*		Control (mm)
	20% ethanol	30% ethanol	20% ethanol	30% ethanol	
A. niger	46.66a	66.66a	46.66a	70.00a	30.00
F.oxysporum	50.00a	70.00a	46.66a	60.00a	30.00
R. stolonifer	47.00a	52.00a	44.11a	52.00a	34.00
B.theobromae	66.66a	66.66a	50.00a	66.66a	30.00
A. flavus	51.62a	73.33a	50.00a	66.66a	30.00
F. solani	50.00a	60.00a	51.62a	53.33a	30.00

Each of the data is a mean of three replacements. Each data followed by the same alphabet along the columns not significantly different at P= 0.05. Using (DMRT) Duncan Multiple Range Test to separate the means.

Discussion.

The radial growth of all the rot fungi: *Rhizopus stolonifer, Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Fusarium solani* and *Botryodiplodia theobromae* were significantly inhibited by the two test plant: *Zingiber officinale* and *Ocimum gratissimum.* This indicates that they have fungitoxic potential, though none of these had 100% inhibition of the radial growth of the mycelia in the Petri dish within the period of observation (Tables and 2).

The results of aqueous and ethanol plant extracts on PDA medium revealed that *Z. officinale* and *O. gratissimum* extracts were mycotoxic to the fungal pathogens but 100% inhibition was not recorded. 30% ethanol extract of *Z. officinale* was found to be more effective than 20% ethanol extract of the same test plant; this was also observed with 30% ethanol extract of *O. gratissium.* Okigbo and Ogbonaya (2006) used leaf extracts of *O. gratissimum* to control post harvest yam rot through cold and hot water, and ethanol extracts. *O. gratissimum* L. is commonly used in folk medicine to treat infection, diarrhea, skin diseases, pneumonia, cough and also conjunctivitis (Onajobi, 1986). It is of family *Leguminoceae*, is grown in garden and used as a tea leaf for fever. It is widely distributed in tropical and warm temperature region (Dalziel, 1937).

Okigbo and Nmeka (2005) used extract of *Z. officinale* to control yam tuber rot. *Z. officinale* Roscoe

family *Zingiberaceae*, is a herbaceous perennial plant which has an upright stems and narrow medium, green leaves arranged in two ranks on each stem. *Z. officinale* or ginger has been used in Asia for relief from arthritis, rheumatism, coughs, fever and infectious diseases (Anonymous 2004 b). Biological control is generally favoured as a method of plant diseases management because it does not have demerit of chemicals (Amadioha and Obi, 1999). Kuhn and Hargreaves (1997) observed that substances found fungicidal in vitro in almost cases kill the fungi *in vivo*. Plants with such fungicidal properties include *Z. officinale* Roscoe (Maurice, 1993).

This work showed that fungitoxic compounds were present in *Z. officinale* and *O. gratissimum* since they were able to control the growth of microbes tested. This agreed with earlier work of some workers on effects of these plants on phyto pathogens of other crops (Amadioha and Obi 1998, 1999). Amienyo and Ataga (2007) used Z. *officinale* extracts to protect mechanically injured sweet potato tubers. Fokunang *et al* (2000) used crude extracts of *Ocimum gratissimum* to control cassava anthracnose diseases. Okigbo *et al* (2005) used *O. gratissimum* against some pathogens of man. Amadioha and obi (1999) also used *O. gratissimum* to control anthracnose diseases of cowpea. The result of this investigation showed that both *Z. officinale* and *O. gratissimum* have potential to control post harvest rot of yam. This can serve as an alternative means of reducing and controlling rot by yam growers and consumers. The pesticides of botanical origin are environmentally non – hazardous by being non phototoxic. Furthermore, the extracts of these botanicals can be easily formulated and applied with little or no literacy of the rural dwellers.

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Testicular maturation and reproductive cycle in mudskipper, *Periophthalmus papilio* (Bloch and Schneider 1801) from Lagos lagoon, Nigeria

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Abstract: A study was carried out on mudskipper, *Periophthalmus papilio* from Lagos lagoon, Nigeria to determine its testicular maturation and reproductive cycle. *P. papilio* is a commercial valued fish in Nigeria as food for man and baits in capture fisheries, making its population in Lagos lagoon to be threatened. Therefore, conservation of its fishery from overfishing and exploitation is urgently required. A total of 796 male individuals were captured with non return valve traps between July 2004 and July 2006 from mangrove swamps of Lagos lagoon. They measured between 37 and 180 (104.83±25.57) mm TL and weighed 1.5 – 60.9 (18.60±10.65) g BW respectively. The testes were morphologically examined by naked eye and processed by standard histological techniques. ICES, BITS and IBTS scales and Bucholtz manuals were employed in the classifications of its maturity and gonadal stages. Seven reproductive stages were encountered in the study viz. immature, immature and developing, ripening, ripe, ripe running, spent and recovering-spent. The reproductive cycle included pre-spawning, spawning and post-spawning phases. The testicular maturation and reproductive cycle in mudskipper, *P. papilio* though with modifications were similar to what obtained in other teleosts. The GSI values ranged between 0.01 and 0.48 (0.132±0.165) i.e. less than 0.48% of the body weight was converted to development of testes. GSI values were at different peaks in July (0.23±0.016) and September (0.30±0.13%) 2004; May (0.198±0.004) and October (0.097±0.009%) 2005; and January (0.865±0.12), April (0.122±0.009) and July (0.145±0.016%) 2006 indicating the species as a multiple and synchronous spawner in Lagos lagoon. The study therefore provides the basic life history information on *P. papilio* through an objective approach in the assignment of maturity stage, using histological technique and macroscopic evaluations of the testes.
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Key words: Gonadosomatic index, spawning, spermatocyte, spermatid, spermatozoon, mudflat

1. Introduction

The present study which investigates the testicular maturation and reproductive cycle in mudskipper, *Periophthalmus papilio* from Lagos lagoon in Nigeria, is a third contribution in the study of reproductive biology of this species. The first and second were documented in Lawson (2010a, Lawson 2010b). Changes in testicular volume, histology, gonadosomatic indexes and other reproductive scores have all been used to document the reproductive cycle, the underlying mechanisms of testicular growth and regression are poorly understood.

P. papilio is a member of Family Periophthalmidae. It is indigenous member of brackish waters of estuarine and creeks. Its Importance lies in its contributions as food to man and baits for artisanal and offshore fisheries. It sells for as high as US$20/kg in Taiwan and Japan (Khaironizam and Norma-Rashid, 2002) and $15 equivalent in Nigeria. Related species in other parts of the world include *P. chrysospilos* and *Boleophthalmus boddaerti* in Singapore, *P. takita* was recently discovered in Australia (Jaafar and Larson, 2008). In Nigeria importance of *P. papilio* has attracted attentions of King and Udo (1996), Etim et al. (1996), Etim et al. (2002), Udo (2002), Lawson 2004a, Lawson 2004b, Lawson 2004c, Lawson 2004d) and Lawson (2011). Little information exists on the reproductive biology of the species especially concerning the males' testicular maturation and reproductive cycle. Aspects of its reproductive biology in Lagos lagoon, Nigeria was reviewed by Lawson (2010a) while the maturation and histological characteristics of ovaries was documented in Lawson (2010b), but made no histological analysis to propose maturity classes for testicular development of this species. However, there are several reviews on testes in some non related teleosts. These include Awaji and Hanyu (1987) on wild type Medaka, *Oryzias latipes*; Ratty et al. (1990) on gonad morphology, histology, and spermatogenesis in Albacore Tuna, *Thunnus alalunga*; in hybrid of Roach, *Rutilus rutilus* and bream, *Abramis brama* by Kopiejewska et al. (2004); Arockiaraj et al. (2004) on freshwater catfish, *Mystus montanus*; El-Greisy (2005) on Brushtooth lizard fish, *Saurida undosquamis*; Santos et al. (2006)

on Characin, *Oligosarcus hepsetus* in Brazilian tropical reservoir; El-Halfawy et al. (2007) on Grey mullet, *Liza ramada* in lake Timsah, Suez canal; and Chakraborty et al. (2007) reports on Sarpunti, *Puntius sarana* in Bangladesh.

Just as the ovary, the testicular maturity can be judged by visual observations by morphological and histological observations (Rath, 2000; Lawson 2010b). Histological study of this species though very strenuous is very essential especially in reproductive system. It is the most accurate method to determine the reproductive state of fish (West, 1990). The present study on testicular maturation of this species by histological investigation provides a basic knowledge of reproductive system in fish and will be a useful tool for further applications in other teleosts.

2.0 Materials and Methods

2.1 Collection of specimens:

A total of 779 individuals (comprising 16 immature and 763 mature males) of mudskipper, *Periophthalmus papilio* were caught from the mudflats of the mangrove swamps of Lagos lagoon (longitude: $3^{o}20'$-$3^{o}50'$W and latitude: $6^{o}24'$-$6^{o}36'$N) between July 2004 and July 2006. The fish were caught with non return valve traps. The diurnal collections were carried out with assistance of artisanal fishermen.

2.2 Laboratory procedures and data collections:

In the laboratory, collection of biometric data such as total length (TL) and body weight (BW) measurements were carried out, TL to the nearest 1 mm and BW to the nearest 0.1 g. The specimens were examined for abnormality or pathological changes and were cut opened through the ventral position. Sexes and gonad maturity stage were ascertained by naked eye examination of the gonads were confirmed under the light microscope. Testes were removed from the specimens, the paired testes were weighed (GW) to the nearest 0.01 g. The testes were fixed in Bouin's fluid. Sections were taken from the middle part of each testicular lobe, dehydrated in various percentages of alcohol, cleared in xylene, and impregnated in paraffin wax between 52-60 ^{o}C melting points. They were embedded in paraffin wax and sectioned at 6 µm thick. The sections were stained in Eirlich haemotoxylin and Eosin (H&E) following Belelander and Ramaley (1979). Microscopic observations of the testes were done under binocular microscope that was mounted with camera and photographs taken. To determine the individual stage of sexual maturation, visual staging of testes was applied. The description of macroscopic criteria was developed by comparing the histological results with the photographic records of the gonads. Maturity stages were evaluated using scales from which each gonad was judged by visual analysis of external features. Sexual maturity of each specimen was classified according to macroscopic scales used in the IBTS (International Bottom Trawl Survey), BITS (Baltic International Trawl Survey), ICES, 1963 (International Council for Exploration of the Sea), (ICES, 1999) and recently, Bucholtz et al (2008) manual, and as well using a microscopic scale, based on histological analysis (Vitale et al., 2005). The microscopic criteria applied in the classification of testicular development were based on testes characteristics such as the formation of spermatogonia, spermatocytes, spermatids, and spermatozoa. This microscopic classification underlines the importance of the passage from endogenous to exogenous spermatocytes, which coincides with the beginning of milt production.

The length at which 50% of the fish population reached sexual maturity (L_{50}) was considered to be the length at first sexual maturity (Pitt, 1970).

The gonadosomatic index (GSI) of the fish was calculated by dividing the ovaries weight by the whole body weight and multiply by 100. Thus:

$$GSI = \frac{GW \times 100\%}{BW}$$

3.0. Results.

3.1 The structure of Testis

The testes were paired and joined posteriorly to form a Y-shaped structure. They appeared as a white multilobed filament and were flat and ribbon like at immature stage. At maturity they appeared creamy, soft, swollen, and multilobed. They lied ventrolaterally to the air bladder and were found in connection with a pair of accessory organ, which increased in size, as the fish grow in size or old.

3.2. Description of testicular maturity stages in *P. papilio*.

3.2.1 Macroscopic characteristics of testes

In the present study, macroscopically the testes were classified into seven (7) stages of maturity which are discussed in Fig. 1.

3.2.1.1. Immature or Stage I:

Specimens classified as immature were those that possessed gonads that were too small to be recognized as males or females. The gonads were too rudimentary in structure to be differentiated. They appeared too small occupying less than $1/10^{th}$ of the abdominal cavity. The naked eye examination did not reveal the presence of accessory sexual organs. Milt not released with gentle pressure.

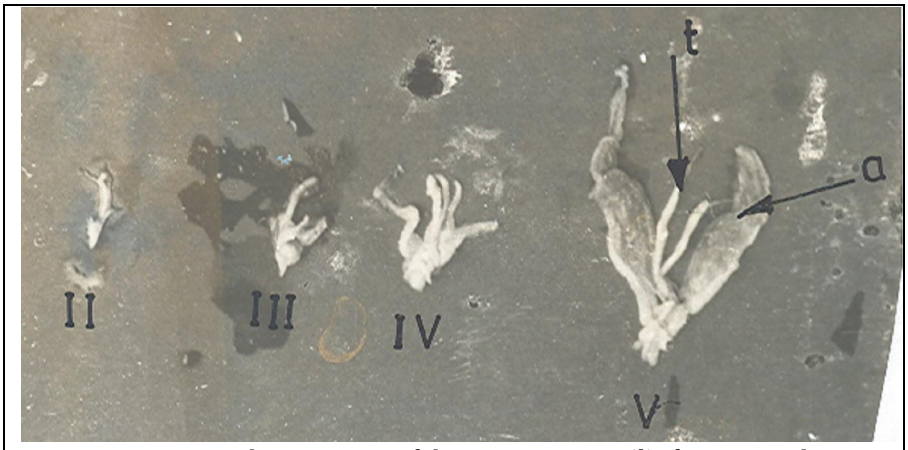


Fig. 1. Macroscopic characteristics of the testes in *P. papilio* from Lagos lagoon, Nigeria.
II, Immature and developing; III, Ripening; IV, Ripe; V, Ripe running; t, testis; a, accessory organ supporting testes.

3.2.1.2. Immature and developing or Stage II:

The testes were flattened and 1-2mm broad, whitish and lobed. They occupied 1% of the body cavity and the accessory sexual organs were rudimentarily visible and appeared small in size. No milt was released with gentle pressure.

3.2.1.3. Ripening or Stage III:

In early stage of ripening, the testes became fatter, off white and occupied 1/8th of the abdominal cavity. Blood vessels were visible through testis wall. Gonad length: width ratio was 2.8. In the late stage, the testes became firm and whiter and occupied 1/5th of the abdominal cavity. Lobulation of the right and left testes started. The length: width ratio was 2.4. The accessory sexual organs were of equal length with the testes. Testes were large but milt not released with gentle pressure.

3.2.1.4. Ripe or Stage IV:

The testes were fully swollen and multilobed at this stage but did not occupy more than 1/4th of the body cavity. The color was creamy white. The accessory sexual organs grew past the testes. The testes were large with milt freely flowing with gentle pressure of the abdomen.

3.2.1.5. Ripe running or Stage V:

The testes were broadest and highly lobulated. They were completely white but the posterior tip sometimes grew with speckled appearance. The accessory sexual organs were fully developed and became longer than the testes. No blood vessels and thick milt exuded on slight pressure before preservation. Testis length: width ratio was 2.2 and it extended for 50% of the abdominal cavity.

3.2.1.6. Spent or Stage VI:

The testes reduced in size and sometimes very small, flaccid and walls were hard in texture. They were dark brown color and no blood vessel visible. Milts were absent or not released with gentle pressure, testis length: width ratio was 3.2 and gonad extended for 30% of the abdominal cavity.

3.2.1.7. Recovering-spent or Stage VII:

Dark patches visible through the testicular wall. The testes were small and about a third of length of body cavity or less and firmer than what obtained at spent stage and 3mm broad.

3.2.2. Comparison of present study with other maturity scales.

Table 1 describes the conversion of the scale developed in this study to the scales of Bucholtz et al (2008), and ICES (1963) and as well as the scales used for the BITS and IBTS surveys. Stages I, II, III, IV, V, VI VII and VIII represent degrees of maturation of the testes generated from the present study based on the stated methodology and as well as scales adapted from ICES, Bucholtz, BITS and IBTS. The ICES scale is commonly used in most laboratories. The ICES, BITS and IBTS and Bucholtz scales were similar except the addition of abnormal stage by Bucholtz covering a stage of reproductive malfunction (stage VIII). However, these scales were modified and simplified in this study for better understanding of the histology of this species. Common to all these scales were a recovering-spent stage which encompassed the final

recovery of the spent gonad as well as the beginning of a new maturation cycle.

Table 1. Comparison of the present scale with other maturity scales currently in use.

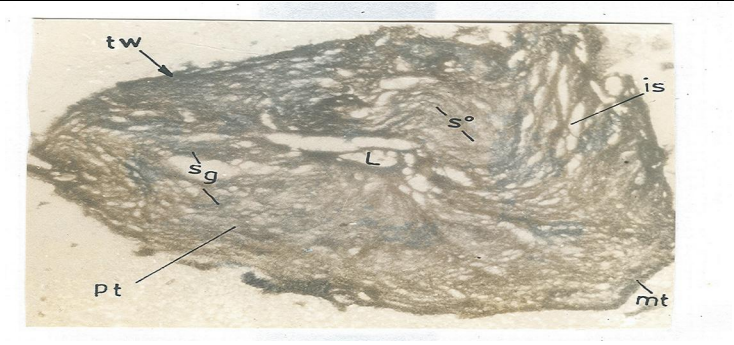
Scale generated from the present study		Current maturity scales in use			
Stage	Degree of maturation	Bucholtz et al 2008	ICES	BITS	IBTS
I	Immature	I. Juvenile	I. Virgin	I. Virgin	I. Immature
II	Immature and developing	II. Early maturation	II. Virgin maturing VII. Recovering- spent	II. Maturing	II. Maturing
III	Ripening	III. Mid maturation	III. Maturing		
IV	Ripe	IV. Late maturation	IV. Maturing		
V	Ripe running	V. Spawning capable	V. Maturing		
VI	Spent	VI. Spawning	VI. Spawning	III. Spawning	III. Spawning
VII	Recovering-spent.	VII. Spent-recovery VIII. Abnormal	VII. Spent	IV. Spent V. Resting	IV. Spent

ICES, International Council for Exploration of the sea; BITS, Baltic International Trawl Survey; IBTS, International Bottom Trawl survey.

3.2.3. Histological characteristics of testes:

The histological characteristics of different maturity stages in the testes of *P. papilio* are presented in Fig. 2.

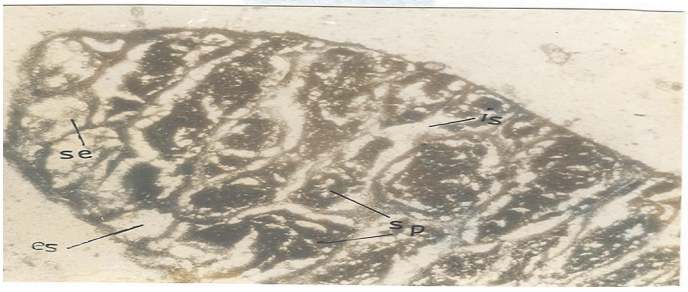
Immature stage: Microscopic examination showed no sexual differentiation. The cells associated with the gonads were rudimentarily developed and could not be differentiated. Hence the specimens were classified as immature. The sample size (N) for this group was 16 specimens.



1000 µm

a: Photomicrograph of T.S of a testis of *P. papilio* in Immature and developing stage

Testicular wall was thick with spermatogonia and primary spermatocytes pre-dominating the peritoneum (Fig. 2a). The mesothelium of the peritoneum was very thick. The stoma and interlobular septa were very conspicuous.
N=313.



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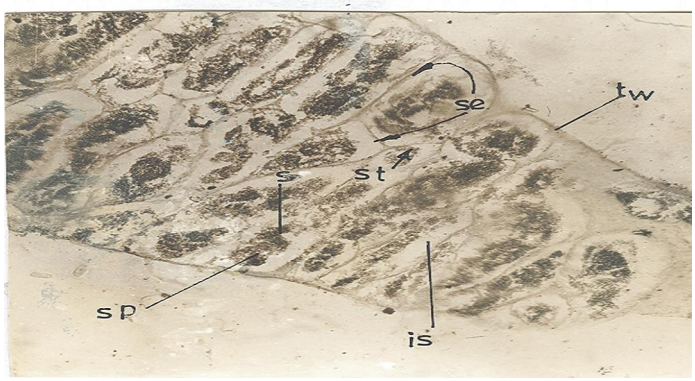
c: Photomicrograph of T.S of a testis of *P. papilio* in ripe stage

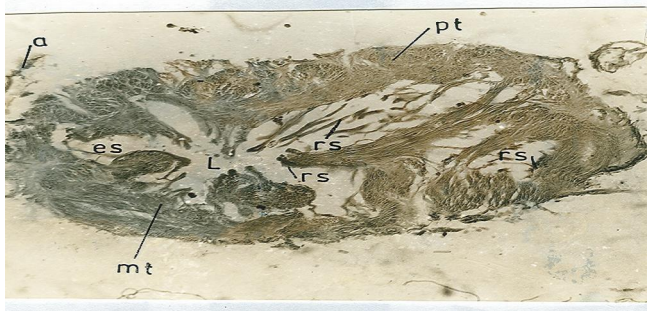
The primary and secondary spermatocytes were dominant while few spermatids and spermatozoa were represented. The testicular wall was 30µm. The testicular septa were well organized and distinct (Fig. 2c).
N=83.



1000µm

d: Photomicrograph of T.S of a testis of *P. papilio* in Ripe running stage.

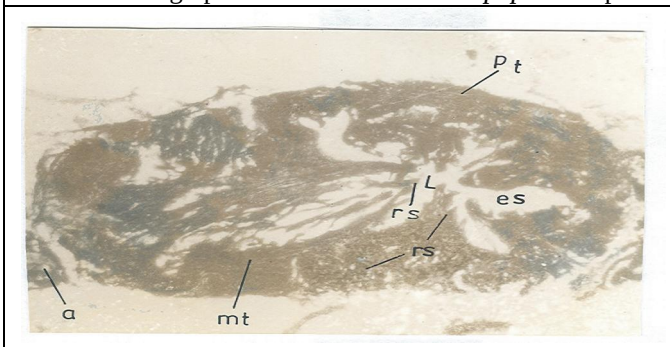
The lumen contained spermatozoa (Fig. 2d). The empty spaces in the lumen also contained spermatocytes and spermatozoa. Most of the spermatozoa migrated towards the periphery of the lobules and primary and secondary spermatocytes and the spermatids were found towards the interior. The testicular wall reached 30µm.
N=111.



1000 µm

e: Photomicrograph of T.S of a testis of *P. papilio* in spent stage

The testis had unfilled lumen with inactive or residual spermatozoa (Fig. 2e) The testicular wall reached 40µm. The accessory sexual organs well developed and longer than what obtained in ripe stage. The septa disappeared and mesothelium was thickest.
N=49.



1000 µm

f: Photomicrograph of T.S of a testis of *P. papilio* in recovering-spent stage.

A big cavity (lumen) was seen at the centre of the gonad and residual spermatozoa were present at the lumen (Fig. 2f). The mesothelium of the peritoneum thickened.
N=5.

Fig. 2a-f. Photomicrographs of Transverse section of testes of *P. papilo* in their different maturity stages in Lagos lagoon, Nigeria.
s^o, primary spermatocyte; is, interlobular septa; sg, spermagonium, pt, peritoneum; mt, mesothelium; L, lumen; es, empty space; st, spermatid; tw, testicular wall; lo=lobule, sp, spermatozoon; se, septum; s', secondary spermatocyte; s, subfollicular space; st, spermatid; tw, testicular wall; es, empty space; pt, peritoneum; a, accessory sex organ; mt, mesothelium; rs, residual spermatozoa; N, sample size.

Histological characteristics of the testes also revealed the process of spermatogenesis in *P. papilio*. The process occurred progressively during the annual reproductive cycle. This study showed five spermatogenic cells (Fig. 3) as follow:

3.2.3.1. Spermatogonia:

Primary and secondary spermatogonia were observed. The former were the largest spermatogenic cell, with clear cytoplasm, large nucleus, occurring isolated. The cysts or nests were absent. The latter were smaller and formed cysts that comprised a few cells.

3.2.3.2 Primary spermatocytes:

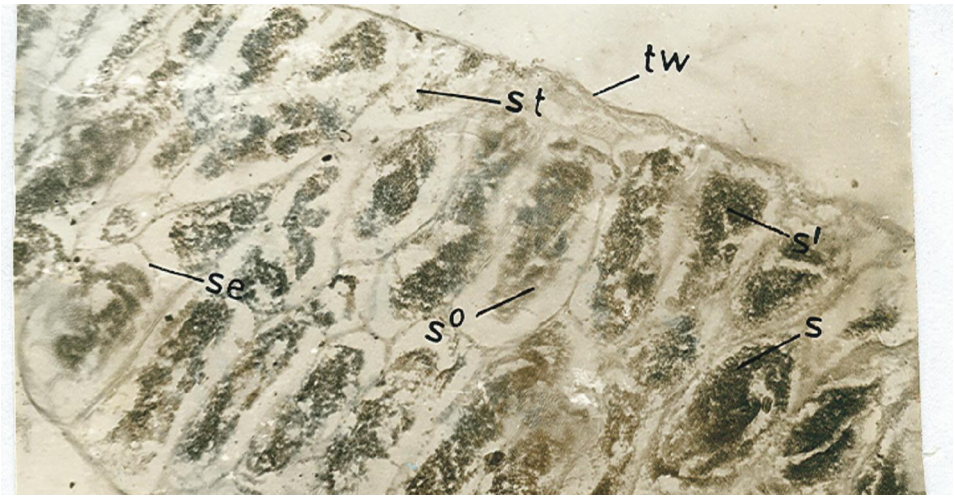
They possessed nuclei that were densely packed with chromatin material. They are product of repeated mitotic divisions of spermatogonia. The prominent nucleus was with filamentous chromatin.

3.2..3.3. Secondary spermatocytes:

They are in large nests that extended into the lobular lumen and characterized by homogenously stained nuclei. They are product of several meiotic divisions. The nucleus has condensed chromatin.

3.2.3.4. Spermatids:

They were smaller in size than secondary spermatocytes. They are product of mitotic division or subsequent maturation of secondary spermatocytes. They were spherical and possessed dense nuclei



1000µm

Fig. 3: A cross section through mature testis of *P. papilio* showing some spermatogenic cells i.e. primary spermatocyte (s^o), secondary spermatocyte (s'), spermatid (st), and spermatozoon (s). tw, testicular wall; se, septum.

.3.2.3.5. Spermatozoa:

These are product of modifications or metamorphosis of spermatid cells. They are the smallest spermatogenic cells. They concentrated in the seminal lobules after breaking through the cyst wall. The sperms later acquired the ability to be mobile in the seminal fluid. This signifies the final product of the process of spermatogenesis.

3.3. Monthly distribution of male *P. papilio* in different maturity stages.

The percentage distribution of different maturity stages in male *P. papilio* is given in Table 2. All stages of maturation were recorded in this study. Stage I testes were encountered in nine (9) of the 25 month(s) duration of study. Percentages varied from 1 in February 2006 and 6.52% in November 2004. Stage II testes occurred throughout the period of study. All male fish in May and June 2005, 2006 were in the immature and developing stage. Stage III, IV and V males showed a similar pattern of distribution occurring throughout the collection period except in May and June 2005, 2006. Stages VI and VII testes appeared in 9 and 4 months respectively. Few numbers were recorded.

3.4. Size at different maturity stages

Table 3 shows that all male fish (stage I testes) with measurements below 60 (48.87±9.92) mm TL and 2.0 (1.57±0.93) g BW were immature, while those (stages II-VII testes) longer than 65 and heavier than 2.2 were considered to be mature males. The mature fish ranged between the mean value of 97.03±15.24 and 139.98±19.35 mm TL weighing 10.53±4.86 to 30.69±10.99 g BW respectively.

The length at first sexual maturity for the male was 65 mm TL and 2.2 g BW.

3.5. Testicular reproductive cycle.

The reproductive cycle in male *P. papilio* was divided into three (3) phases (Fig. 4). The pre-spawning phase included immature, immature and developing and ripening stage testes. In this phase, blood capillaries were not very conspicuous; numerous spermatogonia were observed inside the small seminiferous lobules. Immature stage only occurred once in the life history of this fish.

The spawning phase, comprised testes in their ripe and ripe running stages. All stages of spermatogenesis were seen in their various lobules. The

spermatogonia decreased in number due to intense spermatogenesis. Numerous primary and secondary spermatocytes were visible. The phase was characterized by primary spermatocytes that were smaller than spermatogonia. The secondary spermatocytes were smaller than the primary and were with chromatin. The blood capillaries were conspicuous; the seminiferous tubules were big and full of sperm masses.

The post-spawning phase consisted testes that were at spent and recovering-spent stages. Empty and collapsing seminiferous lobules were observed. Residual or unexpelled sperm were also seen. In addition recovering-spent testes showed developing spermatogonia inside the small seminiferous lobules.

Table 2. Monthly distribution of different maturity stages in *P. papilio* from Lagos lagoon, Nigeria.

Year	Month	Total	Stage I		Stage II		Stage III		Stage IV		Stage V		Stage VI		Stage VII	
			no	%	no	%	no	%	no	%	no	%	no	%	no	%
2004	July	8			1	12.5	1	12.5	5		1	12.5				
	August	10			1	10	3	30	4	40	2	20				
	September	5			1	20	2	40	1	20	1	20				
	October	10			6	60	3	30			1	10				
	November	46	3	6.52	15	32.61	13	28.26	5	10.87	8	17.39	2	4.35		
	December	39	2	5.13	13	33.33	5	12.82	3	7.69	3	7.69	13	33.33		
2005	January	138	2	1.45	53	38.41	41	29.71	14	10.15	20	14.49	8	5.80		
	February	51	2	3.92	17	33.33	18	35.29	4	7.84	9	17.65			1	1.96
	March	29			11	37.93	8	27.59	2	6.90	8	27.59				
	April	6			1	16.67	1	16.67	3	50	1	16.67				
	May	2			2	100										
	June	4			4	100										
	July	10			3	30	5	50	1	10	1	10				
	August	12			5	41.67	4	33.33	1	8.33	8.33		2	16.67		
	September	6			4	66.67	2	33.33								
	October	12			5	41.67	2	16.67			2	16.67	3	25		
	November	86	1	1.16	24	27.91	29	33.72	9	10.47	10	11.63	12	13.95	1	1.16
	December	73	2	2.74	42	57.53	15	20.55	5	6.84	7	9.59	2	2.74		
2006	January	72	2	2.78	40	55.56	17	23.61	3	4.17	9	12.5	1	1.39		
	February	100	1	1.0	38	38.0	28	28.0	12	12.0	14	14.0	6	6.0	1	1.0
	March	40	1	2.5	14	35.0	14	35.0	3	7.5	7	17.5			1	2.5
	April	20			7	35.0	4	20.0	4	20.0	5	25.0				
	May	4			1	25.0	1	25.0	1	25.0	1	25.0				
	June	3			3	100										
	July	10			2	20.0	3	30.0	3	30.0	2	20.0				
Total		796	16		313		219		83		112		49		4	

Table 3. Fish size at different maturity stages in *P. papilio.*

Maturity stage	Range (total length in mm)			Range (body weight in g)		
	min.	max.	Mean±SE	Min	max	Mean±SE
I	37	60	48.87±9.92	1.5	2.0	1.57±0.93
II	65	145	97.03±15.24	2.2	30.9	10.53±4.86
III	76	163	116.68±17.73	5.6	43	18.33±8.68
IV	85	180	127.03±22.49	6.8	60.9	23.30±12.54
V	90	168	139.98±19.35	7.4	52	30.69±10.99
VI	100	165	135.39±18.46	11.2	45.6	26.50±9.42
VII	114	132	124.2±7.36	14.4	28.3	21.4±5.13

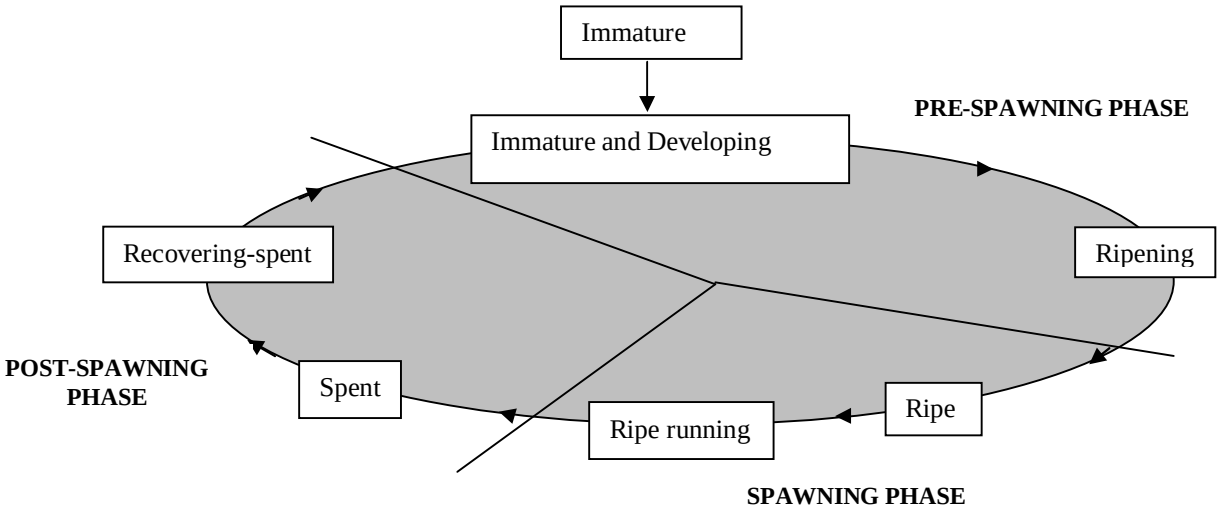


Fig. 4. A schematic diagram of reproductive cycle in male *P. papilio* from Lagos lagoon, Nigeria.

3.6. Frequency distribution of Reproductive phases

The histograms of frequency distribution of reproductive phases of this species are presented in Fig. 5. Of the seven (7) maturity stages and three (3) maturations phases encountered in the study, testes in the stage II were the most abundant constituting 39.32% of the population. The least was stage VII testes that contributed 0.5%. The pre-spawners were more in number than the spawning or post-spawning fish constituting 68.84, 24.50 and 6.66% of the catch respectively.

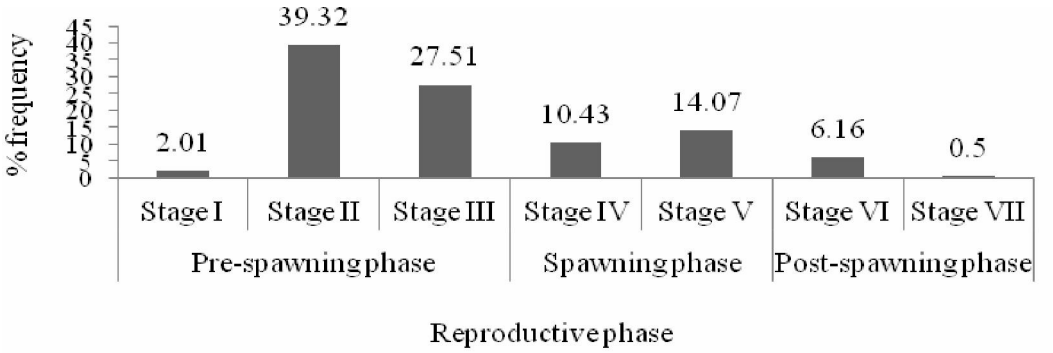


Fig. 5. Reproductive phases in testes of *P. papilio* from Lagos lagoon , Nigeria.

3.7. Gonadosomatic index (GSI) of *P. papilio*.

The monthly GSI values in male *P. papilio* from Lagos lagoon are presented in Table 4. The values varied between 0.01 and 0.48 % in August and September 2004 respectively. The overall mean GSI value was 0.132±0.17%.

The polygons of monthly mean GSI (Fig. 6) revealed significant differences ($P<0.05$) among the different stages of maturity in the fish. The lowest value of 0.02±0.002 % was recorded in August 2005 and 0.865±0.12% in January 2005. The figure showed different peaks of mean GSI in July (0.23±0.016) and September (0.30±0.13%) 2004; May (0.198±0.004) and October (0.097±0.009%) 2005; and January (0.865±0.12), April (0.122±0.009) and July (0.145±0.016%) 2006.

Table 4. The GSI values for male *P. papilio* in Lagos lagoon, Nigeria

Year	Month	Range	
		Minimum	Maximum
2004	July	0.16	0.28
	August	0.01	0.06
	September	0.11	0.48
	October	0.08	0.10
	November	0.04	0.32
	December	0.05	0.12
2005	January	0.034	0.28
	February	0.015	0.217
	March	0.039	0.301
	April	0.104	0.19
	May	0.09	0.28
	June	0.05	0.09
	July	0.041	0.076
	August	0.01	0.045
	September	0.02	0.081
	October	0.084	0.11
	November	0.015	0.102
	December	0.017	0.076
2006	January	0.08	0.189
	February	0.03	0.25
	March	0.03	0.26
	April	0.07	0.25
	May	0.05	0.09
	June	0.02	0.05
	July	0.03	0.09

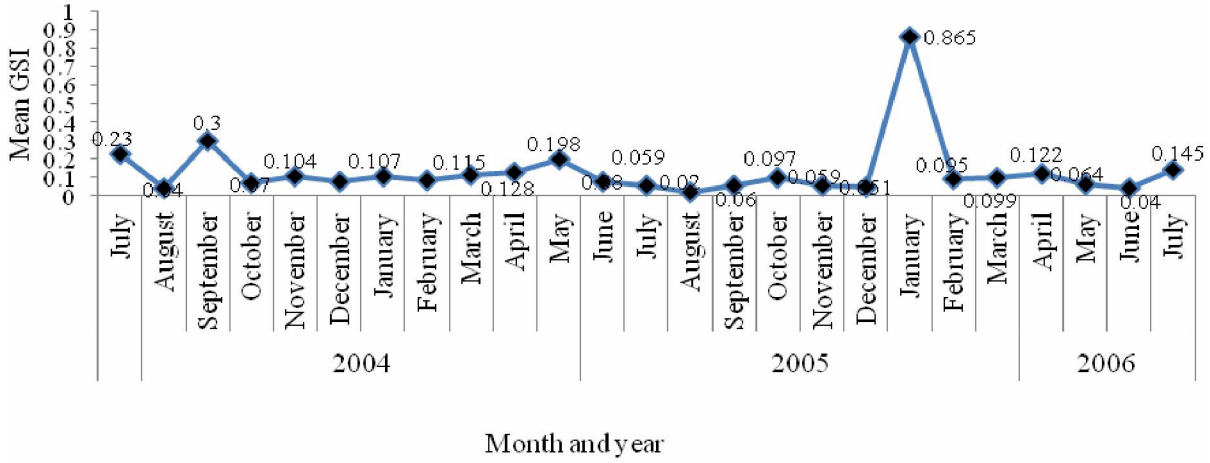


Fig. 6. Monthly mean GSI in testes of *P. papilio* from Lagos lagoon, Nigeria.

4.0. Discussion.

In the present study seven stages of testicular maturation were developed. They are immature (stage I), immature and developing (II), ripening (III), ripe (IV), ripe running (V), spent (VI) and recovering-spent (VII) stages. These were classified into 3 reproductive phases. Stages I-III were testes in pre-spawning phase, IV and V were spawners while VI and VII were classified as post spawning testes. This result was additional data and an improvement over the scales that were generated by ICES (1963, ICES, 1999), BITS, IBTS and Bucholtz et al (2008). A scale generated from the present study was a modification of the ICES, BIT, and IBTS scales that were used in the current study

(Table 1). The general pattern of histological development of the testes of the present study conforms to that of the most teleosts. A 4-stage maturity scale was generated by 1BTS, 5 by BITS, 7 by ICES and 8 by Bucholtz et al (2008) for Herrings and Cods. These scales were reportedly applied in histological study of many teleosts. The maturity stages are hardly discernible by the naked eye and consequently the most susceptible to misclassification.

The histological development of testes showed that cells in immature testes were not discernible to be differentiated as males or females. Those at ripening stage showed active spermatogenesis, while those at ripe and ripe running stages indicated swollen lobules with sperm that were typical of fish in their breeding period. Testes at spent stage presented lobules with residual spermatozoa. Fish in the recovering-spent stage showed testes with spermatogonia and spermatocytes along the wall of seminal lobules, while the lumens of the lobule were with spermatids.

The immature stage can only occur once. Immature and developing signals the entry of the fish into gonadotropin dependent spermatogenesis and gonadal growth. In ripening stage, testes begin to develop not spawn soon. Ripe and ripe running testes indicate that the spermatozoa in lumen are sufficiently advanced and capable of spawning. Spent stage indicates a regressing phase when there was cessation of spawning or completion of spawning season in their burrows. Those in their recovering-spent stage are sexually mature but reproductively inactive. These reports supported and were added data to the study conducted by Brown-Peterson et al (2007). The histological criteria identifying stages of maturity may vary for different species and may not always occur sequentially, but each stage is conceptually universal.

Development of sperm or milt in fish according to Shein et al (2004) passes through multiplication stage, growth and maturation stages. The testes of *P. papilo* consisted of seminiferous tubules, spermatogonium, spermatocyte, spermatids and spermatozoon as were observed in Fig 2 and 3. Their presence was an indication that the gamete or sperm had gone through process of maturation (spermatogenesis). Spermatogonium is a primordial male germ cell that may divide by mitosis to produce primary spermatocyte. The spermatocyte undergoes two meiotic divisions to form four spermatids, which further divisions give rise to spermatozoa.

The length at first sexual maturity of male *P. papilio* in Lagos lagoon was 65 mm TL. Teleosts attain sexual maturity at various ages depending on the species, latitude, water temperature, salinity. The age, at which fish living in a water body under natural environmental conditions (in regard to age and season) attain maturity depends on the latitude. The environmental factors such as temperature, photoperiod, nutrient supply, dissolved oxygen, diseases or parasites are well known to influence reproductive maturity in fish (Cambray, 1994; Joy et al 1999). But the mechanism of action of various environmental factors as well as the sites of their action remains to be determined at the cellular and molecular levels (Lawson 2010b).

The fish burrowed and spawned in the mud flats, this was responsible for fewer populations of the spawners and post spawning fish. Fish close to spawning phase enter the spawning nests and stayed there for some while even at spent stage. This may be reason for the higher percentage of pre-spawners (68.84%) than either spawning (24.50%) or post-spawning (6.66%) fish recorded in the present study. The same trend was reported by Lawson (2010b) in female *P. papilio* in Lagos lagoon. Nest spawning behaviour was reported in *B. pectinirostris*, *P. cantonensis* ,and *P. modestus* by Uchida (1931); and Dotsu and Matob (1977) in Ariake sound and Washio et al (1993) in Midori River, Kumamoto prefecture in Japan. The maturation following their migration to the spawning nest could also responsible for their inability to be collected with gears and traps.

In the present study, there was variation in the monthly GSI values, this according to Washio et al (1993) reports on mudskipper species, *B. pectinirostris* is closely related to the annual changes in reproduction. The highest GSI values correspond to when the testes were at ripe and ripe running stages (spawning phase) while the lowest GSI values indicate totally spent stage. The recovering-spent, immature and developing and ripening stages showed slightly higher mean GSI values than the spent stage. The different peaks of mean GSI values that were observed in the months of July, September, May, October, January and April presumed the fish as a multiple and synchronous spawner in Lagos lagoon. The species spawn several times within a spawning season. The female was reported as a multiple spawner in Lagos lagoon by Lawson (2010b). This probably contributes to reproductive success of the species in Lagos lagoon.

In Lagos lagoon, between 0.01 and 0.48% of the body mass was converted to gonad development by the fish. The GSI varied with species, sex, seasons and availability of food and these were in conformity with reports from other teleosts (Lawson and Aguda 2010; Lawson and Jimoh, 2010; Lawson et al 2010; Lawson, 2011) in some Nigerian waters. The GSI had been used to describe the development of gonads in Pike, *Esox lucius* by Danilenko (1983). It has been widely used as indicator of the fish spawning period, but its use in reproductive biology studies is more suitable when associated with other reproduction indicators such as macroscopic and histological techniques. This is very important in males since differences in size (length and

weight) according to Chaves (1991) are less conspicuous than in females. GSI increases progressively with increases in the percentages of ripe individuals towards the spawning seasons (Mohamed, 2010).

In *P. papilio* GSI values increased from recovering-spent to ripening stage, reaching a peak in ripe or ripe running stage, followed by a decreasing trend to the spent stage. This pattern is expected in teleosts. The most common practice for determination of a species spawning season is the establishment of its GSI and the histological examination of the gonads (El-Greisy, 2000; Assem, 2000 and Assem 2003; Honji et al. 2006).

Therefore, the study has provided information on the testicular maturation process and reproductive cycle of mudskipper, *P. papilio* in Lagos lagoon, Nigeria. The study also contribute baseline data towards management ecology, conservation and biological studies of this and other commercially valued fish in Lagos lagoon complex.

There is an on going research work of the ultrastructural characteristics of the gonads in this species using a transmission electron microscope. The reports of the study will be reported in the next paper.

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Application of an Artificial Neural Network Model to Rivers Water Quality Indexes Prediction – A Case Study

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Abstract: Taxonomic Recent trends in the management of water supply have increased the need for modeling techniques that can provide reliable, efficient, and accurate representation of the nonlinear dynamics of water quality within water distribution systems. Since artificial neural networks have been widely applied to the nonlinear transfer function approximation, in this study we present an empirical multi layer perceptron neural network to estimate water quality indexes (BOD, Do) in Morad Big River in the western part of Iran. In this paper, the information and data including 10 monthly parameters of water quality in the Hamedan Morad Big River in duration of one year and six stations were used for modeling biological oxygen demanded (BOD) and dissolved oxygen (DO) as indices affecting water quality. To validate the performance of the trained ANN, it was applied to an unseen data set from a station in the region. Performance of the model was evaluated by statistical criteria includes correlation coefficient (r), root mean square error (RMSE) and mean absolute error (MAE). In the optimum structure of neural network correlation coefficient for BOD and DO are 0.986 and 0.969, also root mean square error are 8.42 and 0.84 respectively. The results show the identified ANN's great potential to simulate water quality variables.
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Keywords: Artificial Neural Networks; Predicting; Water Quality Index; BOD; DO

1. Introduction

As population and industrial development increases, so does the need for water. The surface water quality in a region largely depends on the nature and extent of the industrial, agricultural and other anthropogenic activities in the catchments. Human beings take water from the hydrologic cycle for their vital and economic needs and give it to the same cycle after using it (Durdu, 2009). The substances that mixed with water during this cycle bring out the concept of water pollution as they change the physical, chemical, and biological properties of water after natural refining (Maier et al, 2004; May and Sivakumar, 2009). In consequence of this, the suitable part of water from which humans cannot cease having for their lives becomes less and less. To prevent this unwanted trend, control of water pollution seriously has become very essential to maintain the sustainability of water resources. River pollution parameters exhibit different properties (Wu et al., 2009; Sahoo et al., 2006). Since the change of pollution in time at any point of a river can affect the downstream of that point at a certain rate, there is a relation also between the change of pollution in time of any point and the change of positional pollution along the river. The river systems are most adversely affected due to their dynamic nature and an easy accessibility for the waste disposal directly or indirectly through drains/tributaries. Since, the rivers and streams are among most important sources of water for irrigation, industrial and other uses, these serve as the lifelines of the population staying in the basins (May et al., 2008). In general, the organic pollution in an aquatic system is measured and expressed in terms of the biochemical oxygen demand (BOD) and declined dissolved oxygen (DO) level. The BOD measures an approximate amount of bio-degradable organic matter present in water and serves as an indicator parameter for the extent of water pollution. The BOD of any aquatic system is the foremost parameter needed for assessment of the water quality as well as development of management strategies for the protection of water resources. This warrants for a foolproof method for its determination. It causes low DO (dissolved oxygen) concentration and unsuitable life conditions for flora and fauna in the river (Dogan et al., 2009). At the same time, BOD–DO relationships include exchange with the river bed and nitrification and denitrification (Sengorur et al., 2006; singh et al., 2009). Nutrients and light in the phytoplankton growth, the relationship between DO and phytoplankton concentrations and ammonia affect the BOD degradation. Currently available method for BOD determination is very tedious and prone to measurement errors. Since, BOD is inversely related to the dissolved oxygen in water, the high values of the earlier indicate for a low level of the dissolved oxygen (DO) or even anoxic conditions in water. Therefore, both these parameters (DO-BOD) are

generally needed to be determined simultaneously and there is a need to devise some suitable secondary (indirect) method for predicting these variables in a large number of samples for water quality assessment (Singh et al, 2009). In recent years, several water quality models such as traditional mechanistic approaches have been developed in order to manage the best practices for conserving the quality of water. Most of these models need several different input data which are not easily accessible and make it a very expensive and time consuming process ANN is the suitable approach for water quality modeling (Chen et al., 2003; Jan-Tai et al., 2006).

The main aim of the present work is to construct an artificial neural network (ANN) model of the Morad Big River water quality (DO,BOD) and demonstrate its application to complex water quality data as how it can improve the interpretation of the results.

2. Material and Methods

2.1. Study Area and Water Quality Data

Morad Big River is in the western part of Iran in Hamedan. The water quality data of 6 stations were used in this study. Those stations located between latitudes 34° 44´ N and 34° 51´ N and longitudes 48° 30´ E. The length of river is 13949 m. The river during its course receives low to very high pollution load from various diffuse and point sources in its different stretches while flowing through urban townships, thus exhibiting very large variations in water quality variables. The locations of these stations are illustrated in Figure 1.

For the stations, the data for September 23 2008 to May 23 2009 were chosen for training and data for May 24 2009 to September 22 2009 were chosen for validation, arbitrarily.

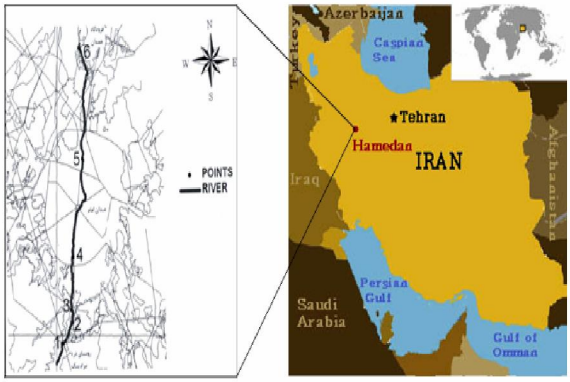

Figure 1. The locations of the stations of Morad Big River (Iran)

At each station, we have measured for 12 different quality parameters are carried out with monthly periods. The measured parameters at these stations in the basin are shown in Table1.

Table 1. Parameters measured at quality observation stations in Morad Big River.

Parameters	Unit
pH	-
Electrical Conductivity	Micromhos/cm
Total Dissolved Solid	mg/lit
Total Suspended Solid	mg/lit
Turbidity	NTU
Sodium	mg/lit
Bicarbonate	mg/lit
Nitrate	mg/lit
Ammoniac	mg/lit
Phosphate	mg/lit
Dissolved oxygen	mg/lit
Biological Oxygen Demand	mg/lit

2.2. Artificial Neural Networks Modeling

In this study, an empirical neural network algorithm was applied to estimate surface water quality parameters (BOD, DO). ANN models are highly flexible function-approximators that have shown their utility in a broad range of water resources applications. Most of these studies showed that ANNs performed better than classical modeling methods (Zhang et al, 2002). ANN is a logical programming technique developed by imitating the working mechanism of human brain. An ANN algorithm can make the brain operations, decide, reach the result in the condition of insufficient data with the help of present information and accept continuous data input, train, and remember. ANN aims to develop the basic operations-which a human brain does biologically-with a definite algorithm. The greatest advantage of a neural network is its ability to model complex nonlinear relationship without a priori assumptions of the nature of the relationship. The ANN model performs a nonlinear functional mapping from the past observations (X_{t-1}, X_{t-2}... X_{t-p}) to the future value X_t, i.e (Durdu, 2009).

$$X_t = f\left(X_{t-1}, X_{t-2}, ..., X_{t-p}\right) + et \quad (1)$$

Where w is a vector of all parameters and f is a function determined by the network structure and connection weights. Thus, the neural network is equivalent to a nonlinear autoregressive model. Training a network is an essential factor for the success of the neural networks. Training the problem by adjusting the weights in itself as using the present information and remembering later are the most important properties of ANN. ANN is a technique that is used in recognizing video and audio, speaking,

analyzing, deciding, defining complex models, and controlling, It differs from the classical programming from very different points of view and is developed by considering working principles of a human brain. In this technique, neuro-physiological working principles (working with electro-chemical principles) of human brains are applied by artificial neurons in computer. The neurons, the basic structural element of a human brain, are electro-chemical operation elements and make operations in degrees of milliseconds. Among the several learning algorithms available, back-propagation has been the most popular and most widely implemented learning algorithm of all neural networks paradigms (Sundarambal et al., 2008; Wu et al., 2008). Feed forward, back propagation networks have previously been identified as the most common type of ANN models used in water resources applications. Therefore, such networks were used in the current study. Networks constructed in the current study comprised of three layers: an input layer, a hidden layer and an output layer. An example of a network topology is shown in Figure 2.

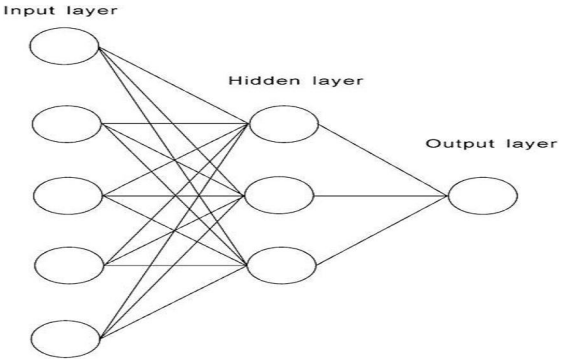

Figure 2. An example of an artificial neural network topology with one input layer, one hidden layer and one output layer

2.2.1. Back Propagation Neural Networks Learning Algorithm

The back propagation (BP) is a commonly used learning algorithm in ANN application (Ying et al, 2007). The back-propagation algorithm based upon the generalized delta rule proposed by Rumelhart et al. (1986) was used to train the ANN in this study. In the back-propagation algorithm, a set of inputs and outputs is selected from the training set and the network calculates the output based on the inputs. This output is subtracted from the actual output to find the output-layer error. The error is back-propagated through the network, and the weights are suitably adjusted. This process continues for the number of prescribed sweeps or until a pre specified error tolerance is reached. The mean square error over the training samples is the typical objective function to be minimized. It uses the back propagation (BP) of the error gradient. This training algorithm is a technique that helps distribute the error in order to arrive at a best fit or minimum error. After the information has gone through the network in a forward direction and the network has predicted an output, the back propagation algorithm redistributes the error associated with this output back through the model, and weights are adjusted accordingly. Minimization of the error is achieved through several iterations. After training is complete, the ANN performance is validated. Depending on the outcome, either the ANN has to be retrained or it can be implemented for its intended use.

In this study, before the training of the network both input and output variables were normalized within the range 0.1–0.9 as follows:

$$x = 0.8\frac{(x - x_{\min})}{(x_{\max} - x_{\min})} + 0.1 \qquad (2)$$

is the normalized value of a certain parameter, x is the measured value for this parameter, xmin and xmax are the minimum and maximum values in the database for this parameter, respectively (Dogan et al, 2009).

2.2.2. Input Variable and Data processing

The monthly data of twelve water quality parameters measured over a period of one year at all the six sampling station were selected for this analysis. The DO and BOD are two major parameters in water quality assessment. Based on existing measured values of different variables and their correlative analysis, total 10 factors (variables) including pH, EC, HCO3, TDS, TSS, Turbidity, NO3, PO4, Na and NH3 were identified which affect the water quality (DO and BOD) to certain degree and finally selected for the model development (table 1). Subsequently, two different ANN models were constructed for the computation of DO and BOD in the river water. The network was trained using the training data set, and then it was validated with the validation data set. The development of ANN models required that the available modeling data be sampled into two smaller subsets for training and validating the network. In this study, the perspective proportions of samples allocated to each of these subsets were as follow:

The data for 23 September 2008 to 23 May 2009 were chosen for training, and data for 24 May 2009 to 22 September 2009 were chosen for validation.

All the computations were performed using the Qnet2000 and EXCEL.

2.3. Performance Criteria

A model trained on the training set can be evaluated by comparing its predictions to the measured values in the over fitting test set. These values are calibrated by systematically adjusting various model parameters. A multi-criteria approach was adopted for assessing the models developed, in which model performance was evaluated using several statistical error and goodness- of-fit measures, including the root mean squared error (RMSE), the mean absolute error (MAE), the correlation coefficient (r). Scatter plots and time series plots are used for visual comparison of the observed and predicted values.

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(P_i - O_i)^2} \quad (3)$$

$$MAE = \frac{1}{n}\sum_{i=1}^{n}|P_i - O_i| \quad (4)$$

$$r = \sqrt{1 - \frac{\sum_{i=1}^{n}(P_i - O_i)^2}{\sum_{i=1}^{n}O_i^2 - \frac{\sum_{i=1}^{n}P_i^2}{n}}} \quad (5)$$

Here P_i and O_i are the predicted and observed values, respectively. And n is the total number of observations.

3. Results

Different ANN models were constructed and tested in order to determine the optimum number of nodes in the hidden layer and transfer functions. Selection of an appropriate number of nodes in the hidden layer is very important aspect as a larger number of these may result in over-fitting, while a smaller number of nodes may not capture the information adequately. Subsequently, two different ANN models were constructed for the computation of DO and BOD in the river water. The network was trained using the training data set, and then it was validated with the validation data set. The optimal network size was selected from the one which resulted in minimum mean absolute error (MAE) in training and validation data sets. The model features for both the ANNs are given in Table 2.

The selected ANN for the DO model is composed of one input layer with ten input variables, one hidden layer with sixteen nodes and one output layer with one output variable, whereas, the BOD model differed in number of nodes in the hidden layer, as it optimized with ten nodes in this layer. The constructed ANN models (DO and BOD) were trained using the Back Propagation algorithm (BP). The correlation coefficient (r), RMSE, and the MAE as computed for the training and validation data sets used for the two models (DO and BOD) are presented in Table 2. Fig.3 shows the plots between measured and model computed values of DO in training and validation sets. The selected ANN (10 nodes in input layer, 16 nodes in hidden layer, and single node in output layer) provided a best fit model for all the three data sets. The correlation coefficient (r) values for the training and validation sets were 0.956 and 0.969 respectively. The respective values of RMSE for the two data sets are 1.02 for training and 0.84 for validation (Table 2). A closely followed pattern of variation by the measured and model computed DO concentrations in river water (Fig. 3), r, RMSE and MAE values suggest for a good-fit of the DO model to the data set.

4. Discussions

In case of the BOD, the selected ANN (10 nodes each in input and 20 nodes in hidden layers and single node in output layer) provided a best fit model for all the two (training and validation) sets. Fig.4 shows the plots between the measured and model computed values of BOD in training and validation sets. The correlation coefficient (r) values for the training and validation sets were 0.969 and 0.986, respectively. The respective values of RMSE for the two data sets are 9.01 for training and 8.42 for validation (Table 2). A closely followed pattern of variation by the measured and model computed BOD values (Fig. 4), r, RMSE and MAE values suggest for a good-fit of the selected BOD model to the data set.

In Fig. 5, the predicted DO and BOD of BPNN with architectures 10-16-1 (ten neurons in the input layer, sixteen neurons in the hidden layer and one neuron in the output layer) and 10-26-1 (ten neurons in the input layer, twenty six neurons in the hidden layer and one neuron in the output layer) are compared with corresponding measured DO and BOD respectively. The figure reveals that an acceptable agreement between the simulations and observations can be achieved. The correlation coefficient values between the ANN models predicted values and observed data for dissolved oxygen and biological oxygen demand are 0.969 and 0.986, respectively, which are satisfactory in common model applications. These results indicate that the neural network model is able to recognize the pattern of the water quality parameters to provide good predictions of the monthly variations of water quality data (BOD and DO) of the Morad Big River.

These results clearly indicate the performance of the neural network. This is expected because of the nonlinear nature of the transfer

function between the water quality characteristics such as BOD, Do and other water quality parameters.

The present study shows that the optimal networks are capable to capture long-term trends observed for the tedious water quality variables (DO and BOD), both in time and space. We propose the neural networks as effective tool for the computation of river water quality and it could also be used in other areas to improve the understanding of river pollution trends. Thus, the ANN can be seen to be a powerful predictive alternative to traditional modeling techniques.

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Table 2. Performance parameters of the artificial neural network models for computation of the DO and BOD in Morad Big River water (Iran)

Model	ANN-Structure	data sets	RMSE (mg/lit)	MAE (mg/lit)	r
DO	10-16-1	Training	1.02	0.78	0.956
		Validation	0.84	0.69	0.969
BOD	10-20-1	Training	9.01	6.71	0.969
		Validation	8.42	6.27	0.986

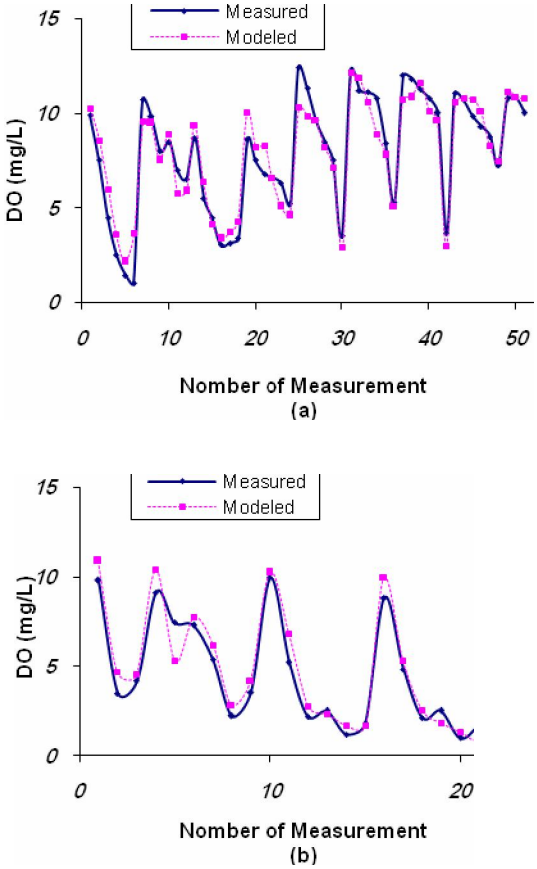


Fig 3. Comparison of the model computed and measured DO levels in the river water (a) training and (b) validation using DO-ANN model.

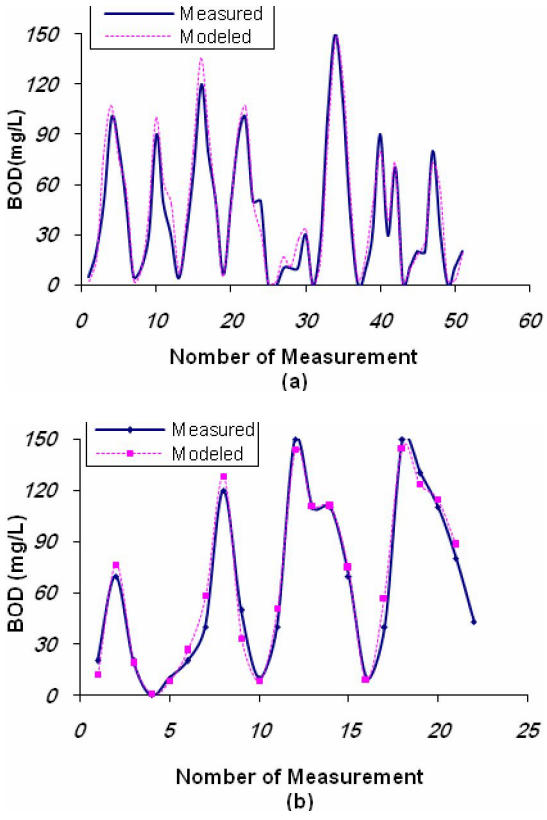
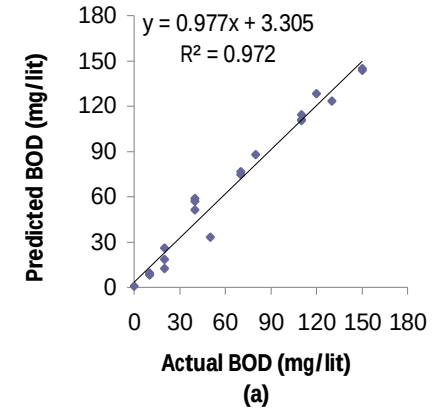


Fig 4. Comparison of the model computed and measured BOD values in the river water (a) training and (b) validation sets using BOD-ANN model



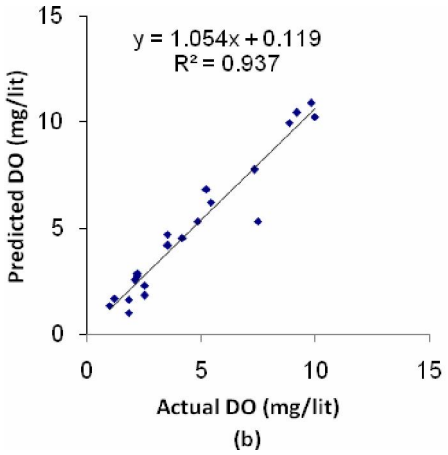


Fig 5. Scatter plots of actual versus predicted BOD values (a) and DO value (b) for the validation data obtained using ANN model.

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Favorable Content of Sustainable Agriculture Extension Programs In Khouzestan Province of Iran

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Abstract: The purpose of research was identify favorable content of sustainable agriculture extension programs in Khouzestan province of Iran. A sample of 79 respondents was selected through simple random sampling technique. A survey study was applied as a methodology of research work. Data were collected using a structured questionnaire that addressed to evaluate agricultural extension experts' responses regarding the necessity of attention on each extension system content to accomplish sustainable agriculture in Khouzestan province of Iran. For determining the validity of questionnaire, the face and content validity was used. Cronbach's alpha was used to measure reliability of the instrument, which was 0.80 and showed the instrument reliability. Descriptive findings revealed that "Food security", "Integrated management", "Biological control practices", "Quality of crops" and "Conservation practices" were the first contents for extension system toward sustainability, respectively. According to factor analysis, the contents of extension system for supporting of sustainable agriculture were categorized into three main components, which have been named *Natural conservation, Human health and Economic contents.* The obtained results from the factor analysis revealed that the three mentioned factors explained 75.231% of the variation of extension content for supporting of sustainable agriculture in agriculture.
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Keywords: Content of extension; Agricultural Sustainability

1. Introduction

In past decades, agricultural development policies have been remarkably successful at emphasizing external inputs as the means to increase food production. This has led to growth in global consumption of pesticides, inorganic fertilizer, animal feedstuffs, and tractors and other machinery (Ommani and Chizari, 2010). These external inputs have, however, substituted for natural processes and resource, rendering them less powerful. Pesticides have replaced biological, cultural, and mechanical methods for controlling pests, weeds, and diseases; inorganic fertilizers have substituted for livestock manures, composts, and nitrogen-fixing crops; information for management decision comes from input suppliers, researchers and extensionists rather than from local sources and fossil fuels have substituted for locally generated energy sources (Roling and Pretty, 1997).

The basic challenge for sustainable agriculture is to make better use of these internal resources. This can be done by minimizing the external inputs used, by regenerating internal resource more effectively, or by combinations of both (Ommani and Chizari, 2007).

According to various studies, the agri-food sector in Iran has not yet shown significant development during the last decades. Despite the advancement of infrastructural services made available to rural people over the past 20 years, they still, live in unequal social and cultural environments. The most important challenges may fall in the following categories: Inadequate resource management for production and insisting degradation of soil or water resources and the associated ecological consequences and inadequate job opportunities (Safaei, 1999). Nevertheless, there is various evidence that agriculture is still far behind the real potential of the country considering its available resource. On the other hand, sustainable land and water use has not yet been reached in Iran (Darvishi, 2003). In Iran, like other developing countries, agriculture is one of the most important economic sectors and comprises a considerably high percentage of production and employment. Rural economic activities are related to three major sectors: agriculture, industry, and services (Ommani et al 2009).

Karshenas (1994) contended that the difficulties within Iranian agriculture have resulted from inefficient resources management by actors within the sector, rather than by a squeeze of natural resources in agriculture. Hence, more consideration to human resources in the agricultural sector is essential. Since farmers and land and water users are the primary active human resources in the agricultural sector, increasing their competence is of necessity to improve the efficiency and productivity of farming. Today more consideration to human resources in the agricultural sector becoming increasingly important because of the competitiveness within the sector. Based on the

research of Karami & Rezaei-Moghaddam (1998), both socio-economic characteristics and environmental conditions of the farm have increased the poverty of Iranian farmers. They suggest that smallholder farmers with under-developed socio-economic and environmental conditions are relatively poorer. They concluded that poverty is a major reason for unsustainable agriculture. Lack of sufficient farm management competencies effectuate higher soil erosion, over-fertilization, inadequate application of manure, lack of fallow, overgrazing, burning of crop residue, and over-use of pesticides.

In addition, Ommani and Chizari (2010) reported that:

"Major barriers hampering adoption of sustainable agriculture practices included: limited financial returns for farmers, limited farmer knowledge of sustainable agriculture principles and methods, low levels of farmer education, government rules and regulations, problems with soil erosion and lack of water, and a low level of extension agent knowledge with respect to sustainable agriculture."

Agricultural extension in Iran such as many developing countries is mainly focused on common extension approach**.** Studies showed that traditional extension system have not been sufficiently effective in promoting adoption of sustainable agriculture practices**.** Studies indicated that Iran's sustainable agricultural extension contents are not favorable and the extension system does not pay enough attention to them. These conditions necessitate rethinking of extension contents to accomplish sustainable agriculture (Ommani and Chizari, 2010., Allahyari, 2008).

The purpose of the present study was to identify the most appropriate contents for agricultural extension toward sustainability in Iran context.

2. Material and Methods

The research method was quantitative research. In quantitative research, the researcher identifies variables and may look for relationships among them, but does not manipulate the variables (Gay and Airasian, 2003). A major form of nonexperimental quantitative research that has been used in this research is correlation study. This method seeks to determine relationships among two or more variables (Creswell, 2008). The total population of agricultural extension experts (N=110) of Agricultural-Jihad Organization of Khouzestan Province, Iran considered as population of study. Based on Krejce and Morgan (1970), 79 of agricultural extension experts selected as sample size of research. A mailed questionnaire was used to collect the data. The model of questionnaire derived from studies of Arellanes and Lee (2003); Hersman (2004); Boone et al (2007), Karami and Rezaei-Moghadam (1998). To test the validity of a questionnaire, content-related evidence of validity by panel of experts was used. To test the content-related evidence, 20 copies were provided and distributed among faculty members of Islamic Azad University. Their suggestions were incorporate in the final version of the instrument. Researchers examined reliability evidence by 30 copies of questionnaire of experts that provided and distributed among agricultural extension experts from Esfahan Province. Reliability of overall instrument was estimated at 0.80. The instrument consisted of two separate sections according to the purpose and objectives of the study. The first section was designed to gather data on personal characteristics of extension experts. The second section was designed to gather data regarding the necessity of attention on each extension system contents to accomplish sustainable agriculture in Iran. Extension experts were asked to rate their viewpoints concerning this necessity on a five point Likert - type scale: 1 = very low, 2 = low, 3 = medium, 4 = much and 5 = very much.

The data were collected between October 2008 and March 2009. After gathering and encoding information from the questionnaires, data was obtained for analysis. Data collected were analyzed using the Statistical Package for the Social Sciences (SPSS, 16). Beside descriptive statistics, Factor Analysis and Kruskal -Wallis test were employed for detailed analysis. Figure 1 indicates, different items were considered in literature of research.

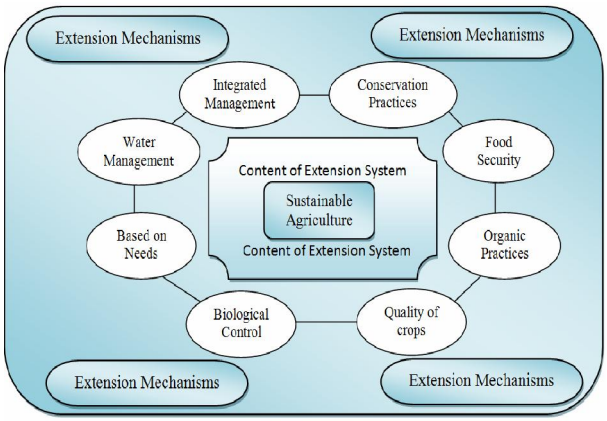

Figure 1: Theoretical Framework of Research

3. Results

The ages of the respondents ranged from 27-58. The mean age was 33 (SD = 7.45, n = 79). The majority (44.3%, n = 35) of respondent were 31-40 years old. The years of experience of respondents ranged from 1-28. The mean years served in extension were 10.8 (SD = 6.54).

In the present study the experts were questioned about the importance rate of extension contents for supporting sustainable agriculture by 5-point scale (1=very low, 2=low, 3=moderate, 4=high, 5=very high). As Table 1 indicates, the five most important extension contents according to the experts were: (1) Food security (M= 3.9, Sd= 1.09), (2) Integrated management (M=3.8, Sd= 1.09), (3) Biological control practices (M= 3.7, Sd=1.11), (4) Quality of crops (M=3.5, Sd= 1.21), and (5) Conservation practices (M= 3.1, Sd= 1.19).

In reference to the frequency of respondents about extension contents, 25.3% of respondents stated that the considering food security had very high importance for supporting sustainable agriculture.

To categorize content of extension systems toward sustainability, an exploratory factor analysis was conducted for the data presented in Table 2. The factor analysis used was a principal components analysis with factor extraction and VARIMAX rotation. The four commonly used decision rules were applied to identify the factors (Hair et al, 2005): 1) Minimum eigenvalue of 1; 2) minimum factor loading of 0.5 for each indicator item; 3) simplicity of factor structure; and 4) exclusion of single item factors.

Based on the results of Bartlett and KMO(Kaiser-Mayer-Olkin) tests was realized whether the data are appropriate for factor analysis (KMO=0.722; Bartlett=832.8, Sig= 0.000). It revealed that the internal coherence of the data is appropriate.

The contents of extension system for supporting of sustainable agriculture were categorized into three main components, which have been named *Natural conservation, Human health and Economic contents* (Table 2). The obtained results from the factor analysis revealed that the three mentioned factors explained 75.231% of the variation of extension content for supporting of sustainable agriculture in agriculture (Table 2, 3). The first group, which is labeled Natural conservation content, consists of four items and Cronbach's alpha for this group is 0.85, which is more than sufficient. This factor had the most Eigen value (3.73). Also, this factor explained 37.574% of the total variances of the variables. The second group, labeled Human health content, is comprised of three items. This component has a Cronbach's alpha of 0.752, which can be regarded as sufficient. In addition, this component that its Eigen value was 2.23 explained 25.371% of the total variances of the variables (Table 2). Based on results field frame work was showed in figure 2.

Table 1. Importance of extension system contents for supporting sustainable water resources management in agriculture

Content of Extension System	Very Low		Low		Average		High		Very High		M	SD	CV	R
	f	*%*	*f*	*%*	*f*	*%*	*f*	*%*	*f*	*%*				
Food security	0	0	3	3.8	21	26.9	35	44.3	20	25.3	3.9	1.09	0.281	1
Integrated management	0	0	5	6.3	26	32.9	28	35.4	20	25.3	3.8	1.09	0.287	2
Biological control practices	2	2.5	10	12.7	22	27.8	23	29.1	22	27.8	3.7	1.11	0.296	3
Quality of crops	7	8.9	11	13.9	21	26.6	15	19	25	31.6	3.5	1.21	0.345	4
Conservation practices	9	11.4	13	16.4	29	36.7	15	19	13	16.4	3.11.19		0.380	5
Water resources efficiency and productivity	7	8.9	15	19	24	30.3	21	26.6	12	15.9	3.2	1.26	0.393	6
Integrating indigenous and new knowledge	12	15.2	20	25.3	21	26.6	15	19	11	13.9	2.9	1.20	0.412	7
Considering crop yield	15	19	17	21.5	32	40.5	12	15.2	3	3.8	2.6	1.15	0.437	8
Mechanical and farming control	12	15.2	23	29.1	29	36.7	11	13.9	4	5.1	2.6	1.29	0.487	9

M=Mean, SD=Standard Deviation, CV= Coefficient of Variation, R=Rank

Table 2. Percent of explained variance by factors underling extension contents

Factors	**Percentage**	**Cumulative Percentage**
Natural conservation content	37.574	36.514
Human health content	25.371	62.918
Economic content	12.313	75.231

Table 3. Rotated component matrix for the extension contents for supporting of SWRM

Content of Extension	Factor Loadings for Components[a]		
	Natural conservation content	Human health content	Economic content
1. Conservation practices	0.781		
2. Integrated management	0.908		
3. Biological control practices	0.875		
4. Mechanical and farming control	0.834		
5. Quality of crops		0.706	
6. Water efficiency and productivity			0.706
7. Integrating indigenous and new knowledge		0.805	
8. Considering crop yield			0.685
9. Food security		0.756	

[a] factor loading< 0.5 were omitted

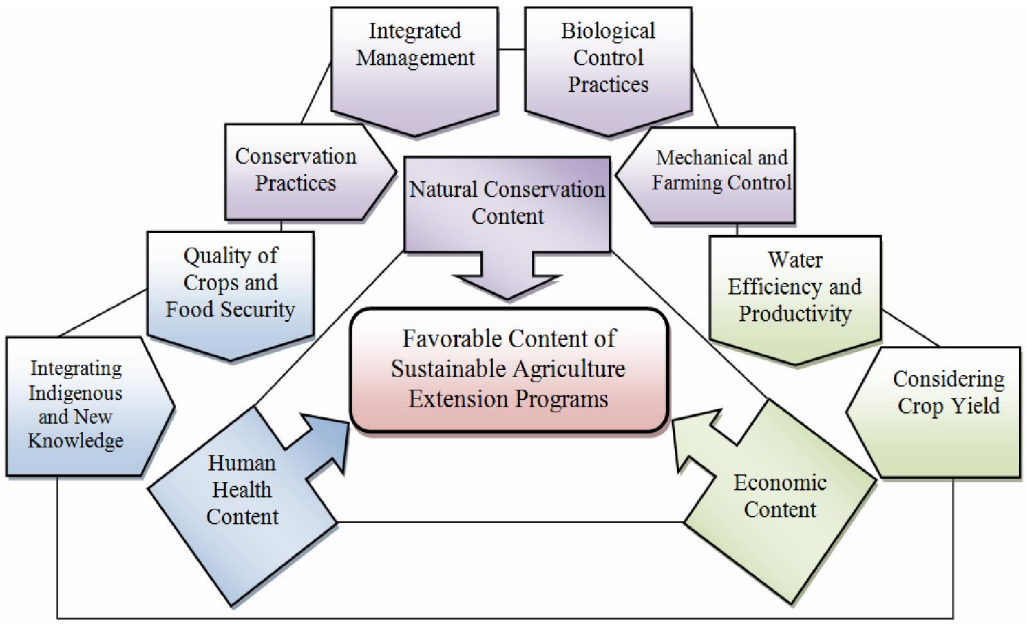

Figure 2: Field Framework of Research

4. Conclusion

For receiving favorability in content of sustainable agriculture extension programs in Khouzestan province of Iran, there is a need for reorientation in content of agricultural extension system. Iran's agriculture is facing serious environmental pollution and degradation problems and extension has a key role to improve it, but current extension system in Iran does not has a sufficient competency for the achievement of sustainability and it needs to shift toward new approaches with new objectives(Ommani and Chizari, 2010., Allahyari, 2008). According to the results of research, contents of extension system for supporting of sustainable agriculture were categorized into three main components, which have been named *Natural conservation, Human health and Economic contents*. Also, five most important extension contents for supporting of sustainable agriculture according to the experts were: (1) Food security, (2)Integrated management, (3)Biological control practices, (4)Quality of crops and (5)Conservation practices.

Because the natural environment strongly influences educational planning and operations, extension should respond to the technological needs of farmers in different agroecological zones. Contents of the agricultural extension should include a broad concept, such as farmers' communication among

each other, informal agricultural education, etc. Farmers strongly require new knowledge to improve their decision skill when they face a series of challenges in the market economy.

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10/23/2010

GC/MS Determination of Bioactive Components of *Murraya koenigii*

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Abstract: In this study, the bioactive components of *Murraya koenigii* leaves have been evaluated using GC/MS. The chemical compositions of the ethanol extract of *Murraya koenigii* were investigated using Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. GC/MS analysis of ethanol extract of *Murraya koenigii* revealed the existence of 1-Methyl-pyrrolidine-2-carboxylic acid (69.00%), Ethyl à-d-glucopyranoside (13.36%), Isolongifolene, 4,5-dehydro- (3.68%), ç-HIMACHALENE (2.88%), 1,2-Ethanediol, monoacetate (2.79%) 1,2-Benzenedicarboxylic acid, diisooctyl ester (2.55%). The results of this study offer a platform of using *Murraya koenigii* as herbal alternative for the current synthetic antimicrobial agents.
[Hema R., S. Kumaravel and K. Alagusundaram. **GC/MS Determination of Bioactive Components of *Murraya koenigii***. Journal of American Science 2011;7(1):80-83]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: *Murraya koenigii*, GC/MS, Bioactive components

Introduction

Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties (de-Fátima *et al*., 2006). Distinguished examples of these compounds include flavonoids, phenols and phenolic glycosides, saponins and cyanogenic glycosides (Shahidi 2000 and Shahidi, *et al*., 2008). Natural products from microbial sources have been the primary source of antibiotics, but with the increasing recognition of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very significant because these may serve as talented sources of book antibiotic prototypes (Meurer-Grimes *et al*., 1996; Koduru *et al*., 2006). It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations (Mathekaga and Meyer, 1998).

Murraya koenigii is an aromatic leaf often used in Indian cuisine. is a tropical to sub-tropical tree in the family Rutaceae, which is native to India. The name itself in Tamil is pronounced as 'kariveppilai' (kari-curry, veppu- neem and ilai-leaf) which is the literical translation of curry leaves. The Tamil name means "leaf that is used to make curry" and present in almost all the dishes of Tamil nadu in addition to coriander leaves, a state of south India. Often used in curries, the leaves generally go by the name "curry leaves", though they are also called "sweet neem leaves." It is an unavoidable content of curries in South India, where without curry leaves, curry seems to be tasteless Curry leaves are also entirely unrelated to bay leaves and basil leaves, which are aromatic leaves from the Mediterranean.

It is a small tree, growing 4-6 m tall, with a trunk up to 40cm diameter. The leaves are pinnate, with 11-21 leaflets, each leaflet 2-4 cm long and 1-2 cm broad. They are highly aromatic. The flowers are small, white, and fragrant. The small black shiny berries are edible, but their seeds are poisonous.

The leaves are highly valued as seasoning in which it is usually fried along with the chopped onion in the first stage of the preparation. In their fresh form, they have a short shelf life, and they don't keep well in the refrigerator. They are also available dried, though the aroma is largely inferior. Although most commonly used in curries, leaves from the curry tree can be used in many other dishes to add spice.

The leaves of *Murraya koenigii* are also used as a herb in Ayurvedic medicine. Their properties include much value as an anti-diabetic (Arunselvan *et al*., 2006; Yadav et al., 2002; Vinuthan *et al*., 2004; and Achyut *et al*., 2005), antioxidant (Arunselvan *et al*., 2007; Vinuthan *et al*., 2004; Singh *et al*., 1978; Goutam *et al*., 1974; Deshmukh *et al*., 1986; Baliga *et al*., 2003), antimicrobial (Abhishek Mathur *et al*., 2010; Vinuthan *et al*., 2004; Singh *et al*., 1978; Goutam *et al*., 1974; Deshmukh *et al*., 1986; Baliga *et al*., 2003), anti-inflammatory (Muthumani *et al*., 2009), hepatoprotective (Pande *et al*., 2009), anti-hypercholesterolemic (Iyer *et al*., 1990 and Khan *et al*., 1996), as well as efficient against colon carcinogenesis (Iyer *et al*., 1990) etc. Curry leaves are also known to be good for hair, for keeping it healthy and long.

Materials and Methods

Plant material and extraction procedure

Leaves of *Murraya koenigii* were bought fresh from local market, Thanjavur. 10gm powdered plant material was soaked in 20ml of Absolute alcohol overnight and then filtered through a Whatman® No. 41 filter paper (pore size 20 - 25 _m) along with 2gm Sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with absolute alcohol. The filtrate is then concentrated by bubbling nitrogen gas into the solution and was concentrated to 1ml. The extract contains both polar and non-polar phytocomponents.

Gas Chromatography–Mass Spectrometry (GC/MS) analysis

GC/MS analysis of this extract was performed using a Perkin Elmer GC Claurus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC/MS) equipped with a Elite-1 fused silica capillary column (30 m × 0.25 mm ID. ×1 µMdf, composed of 100% Dimethyl poly siloxane). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min. and an injection volume of 2 µl was employed (split ratio of 10:1). Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min. isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min.The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a TurboMass Ver5.2.0

Results and Discussion

Identification of Components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The Name, Molecular weight and Structure of the components of the test materials were ascertained.

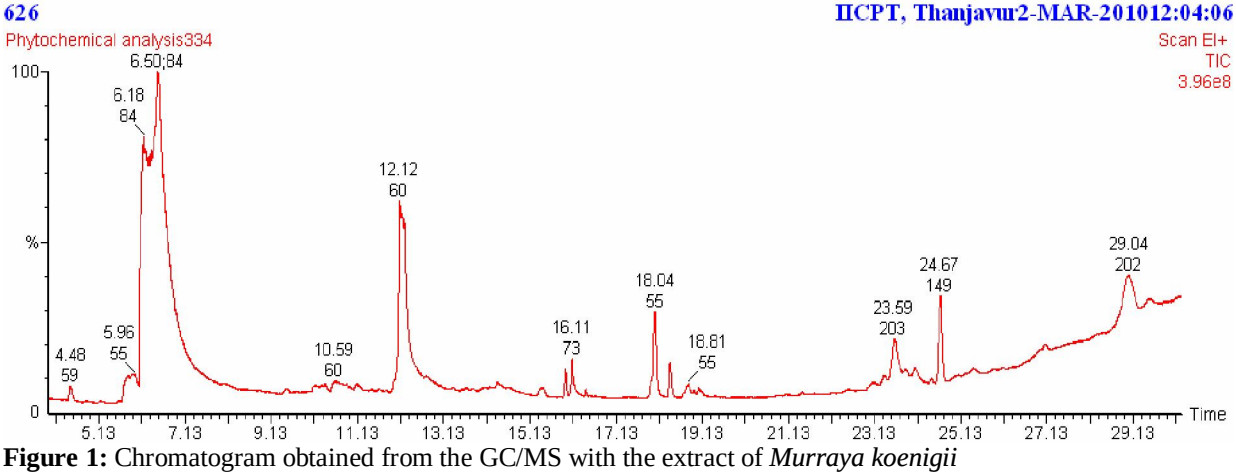

Figure 1: Chromatogram obtained from the GC/MS with the extract of *Murraya koenigii*

Table 1. Total ionic chromatogram (GC–MS) of ethanol extract of *Murraya koenigii* obtained with 70 eV using a Elite-1 fused silica capillary column with He gas as the carrier.

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	4.48	Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	176	0.56
2	5.96	1,2-Ethanediol, monoacetate	$C_4H_8O_3$	104	2.79
3	6.50	1-Methyl-pyrrolidine-2-carboxylic acid	$C_6H_{11}NO_2$	129	69.00
4	12.12	Ethyl à-d-glucopyranoside	$C_8H_{16}O_6$	208	13.36

5	15.41	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270	0.39
6	16.11	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.81
7	16.43	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.11
8	18.04	Oleic acid, methyl ester	$C_{19}H_{36}O_2$	296	2.54
9	18.39	Phytol	$C_{20}H_{40}O$	296	0.72
10	18.81	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	0.60
11	23.59	ç-HIMACHALENE	$C_{15}H_{24}$	204	2.88
12	24.67	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	2.55
13	29.04	Isolongifolene, 4,5-dehydro-	$C_{15}H_{22}$	202	3.68

Thirteen compounds were identified in *Murraya koenigii* leaf extract by GC-MS analysis .The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and Concentration (%) are presented in (Table 1 and Fig 1).The prevailing compounds were 1-Methyl-pyrrolidine-2-carboxylic acid (69.00%), Ethyl à-d-glucopyranoside (13.36%), Isolongifolene, 4,5-dehydro- (3.68%), ç-HIMACHALENE (2.88%), 1,2-Ethanediol, monoacetate (2.79%) 1,2-Benzenedicarboxylic acid, diisooctyl ester (2.55%).

Table 2: Major Phyto-components and its biological activities obtained through the GC/MS Study of *Murraya koenigii* have been listed

Sl. No.	Retention Time	Peak Area %	Name of the Compound	Active biological activity
1.	6.50	69.00	1-Methyl-pyrrolidine-2-carboxylic acid	Used in the formulation of drugs by both oral and transdermal delivery routes
2.	12.12	13.36	Ethyl à-d-glucopyranoside	Preservative
3.	16.11	0.81	n-Hexadecanoic acid	Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-Alpha reductase inhibitor
4.	16.43	0.11	Hexadecanoic acid, ethyl ester	Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-Alpha reductase inhibitor
5.	18.04	2.54	Oleic acid, methyl ester	5-Alpha-Reductase-Inhibitor, Allergenic, Alpha-Reductase-Inhibitor, Anemiagenic, Antialopecic, Antiandrogenic, Antiinflammatory, Antileukotriene-D4 (Anti-platelet activating factor), Cancer-Preventive, Choleretic, Dermatitigenic Flavor, Hypocholesterolemic, Insectifuge Irritant, Percutaneostimulant, Perfumery, Propecic
6.	18.39	0.72	Phytol	Cancer-Preventive
7.	18.81	0.60	9,12-Octadecadienoic acid (Z,Z)-	Antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge
8.	23.59	2.88	ç-HIMACHALENE	Used in flavouring of spirit drinks
9.	24.67	2.55	1,2-Benzenedicarboxylic acid, diisooctyl ester	Used as Softeners, Used in preparation of perfumes and cosmetics, Used as plasticized vinyl seats on furniture and in cars, and clothing including jackets, raincoats and boots. Used in textiles, as dyestuffs, cosmetics and glass making.
10.	29.04	3.68	Isolongifolene, 4,5-dehydro-	Anti-proliferative

The biological activities listed are based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

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Stainless steel implantation-induced changes in surface characteristics, corrosion resistance and hemato-biochemical parameters of male rat

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Abstract: In this study the physiological solution effect on corrosion resistance and surface characteristics of stainless steel has been studied *in vitro* by electrochemical measurements and microstructure characterization of the surface. All studies were carried out using phosphate buffer saline (PBS) as a simulated physiological solution. Potentiodynamic polarization results indicated a considerable shift of pitting potential of the specimen in the noble direction after14 days of immersion in PBS. As evidenced by electrochemical impedance spectroscopy (EIS), the effect of long immersion of stainless steel in physiological solution on the passive film stability was proved. The surface structure and composition before and after immersion in PBS were then characterized by means of scanning electron microscopy (SEM) with electron diffraction X-ray analysis (EDX) techniques. The electrochemical measurements and fitting parameters showed that the passive film formed on stainless steel decreased the corrosion currents densities (I_{corr}) and the constant phase elements (*CPE*), as simultaneously increased the values of polarization or charge transfer resistance (R_{ct}) of stainless steel in simulated physiological solution. The physiological and histological effects of pitting corrosion of stainless steel metal were studied after 14 days of post-implantation in the tibiae of Sprague-Dawley male rats. The stainless steel implantation caused a slightly increased in blood haemoglobin, total erythrocytes count and packed cell volume, and significantly decreased total leukocyte count. All the hepatic enzymes activities of a separate aminotransferase, alanine aminotransferase, alkaline phosphatase and lactate dehydrogenase were significantly decreased. The activity of glutathione S-transferase and the level of lipid peroxidation were significantly increased while hepatic glutathione was significantly decreased. The toxicity of stainless steel in implanted rat could be related to the biodegradation of the alloy and releasing of Fe, Mn, Ni and Cr in the rat tissue as indicated by the *in vitro* study. The bone regeneration was observed at the surface near the stainless steels implants after two weeks of implantation.

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***Keywords*:** Impedance spectra; Pitting corrosion; Scanning electron microscope (SEM); Electron diffraction X-ray (EDX) analysis; Lipid peroxidation; Glutathione; Toxicity; Bone repair.

1. Introduction

Biocompatibility is the ability of a material to perform with an appropriate host response in a specific application. This means that the tissue of the patient that comes into contact with the materials does not suffer from any toxic, irritating, inflammatory, allergic, mutagenic, or carcinogenetic action. Hence the attention of researchers to study the corrosion susceptibility of various metals and alloys used in surgical implantation in the physiological fluids (Baroux, 1993). Surgical implants are usually made of metallic materials, such as titanium and its alloys, stainless steels and cobalt - chromium alloys. Among all the metallic materials, stainless steel is the most popular because of their relatively low cost, ease of fabrication and reasonable corrosion resistance. However, stainless steel is susceptible to a number of localized corrosion, such as pitting and crevice corrosion, intergranular corrosion (IGC) and stress corrosion cracking (SCC) (Shaikh et al., 2006). A number of failures of stainless steel materials during its implantation have been reported (Rondelli et al., 2005); due to their high nickel (Ni) content and to the aggressive biological effects. The corrosion products include iron, chromium, nickel and molybdenum. Although new Ni-free stainless steels has been subjected to biological studies and shown promising results (Fini et al., 2003) until now many of the developing countries still use commercial stainless steel which contains Ni element particularly in the filed of bone surgeries. Stainless steel implants are used as temporary implants to help bone healing, as well as fixed implants such as for artificial joints. Typical temporary applications are plates, medullar nails, screws, pins, sutures and steel threads and networks used in fixing fractures (Virtanen et al., 2008). Although stainless steel is seldom used in developed countries as permanent implants, it is still the most used in emerging countries (Ballarre et al., 2010). Stainless steels are iron-base alloys with a minimum of 10.5% Cr as an alloying element, needed to prevent the formation of rust (Haritopoulos et al., 2007). The susceptibility of stainless steel to the different

types of corrosion, especially pitting corrosion depends primarily on the environmental parameters besides the chemical composition and metallurgical manufacturing condition of the steels. The effects of various anions present in surrounding environment on the pitting of stainless steel have been studied by many authors. Zuo et al (2002); reported the inhibition effects of OH^-, NO^{3-}, SO_4^{2--}, ClO_4^- and acetate ions on pitting of stainless steel in chloride solutions. An increase of Cr content strongly increases the resistance against localized breakdown of passivity. Reliable prediction of the corrosion behaviour is the fundamental step towards effective control of corrosion. Electrochemical measurements involving electrochemical polarization and electrochemical impedance spectroscopy techniques were performed in physiological solutions in order to determine and compare the corrosion behaviour of the different implanted materials under variable conditions (Hiromoto et al., 2002). Electrochemical polarization methods are classified as controlled potential (potentiostatic, potentiodynamic) and controlled current (galvanostatic) approaches. The polarization curve associated with a potentiodynamic method allows detailed study of the important parameters that impact the formation and growth of passive films (E_{corr}) and pit propagation (E_{pit}). This method was successfully used to explain the pitting and passivation on stainless steel. On another hand, EIS has been successfully used to investigate the corrosion and passivation phenomena (Fadl-Allah et al., 2008). It enables the direct matching of the electrochemical system to equivalent circuit models. These equivalent circuits consist of discrete electronic components, resistor, capacitors and/or inductors, which can describe the properties of the electrochemical system under investigation.

Despite the high corrosion resistance of stainless steels and good mechanical properties, but corrosion occurs promoting the release of metal ions that penetrate into the biological tissues. The ions that released are disseminated throughout the body and partially accumulated in the liver, kidneys and spleen. Surface characterization of these metallic alloys is highly important as a tool to evaluate the performance of the implant through the interaction surface film-tissue and the possible migration of metallic ions from the base metal to the nearby tissue. The analysis of *in vivo* formation of new tissue at the interfaces of bioactive implants has been reported using histological methods and the interfacial mechanical.

Therefore, the present study was carried out *in vitro* to (1) provide an improved understanding of the corrosion process on stainless steel when soaked in simulated physiological solution which is prepared by dissolving only inorganic components, (2) characterize the relationships between corrosion behaviour and surface characteristics of stainless steel before and after two weeks from its immersion in simulated physiological solution by using scanning electron microscopy (SEM) to study the surface morphology and electron diffraction X-ray analysis (EDX) to analyze the chemical composition of the surface. Moreover, the aim of this study was to evaluate physiological and histological effect of pitting corrosion of local stainless steel metal in osteosynthesis of the body. Some haematological parameters and the alterations in the levels of glutathione, lipid peroxidation and some enzyme activities of liver tissues of male rat were determined after two weeks of post-implantation of commercially stainless steel laminar in tibiae of rat.

2. Materials and methods

2.1 Materials and chemicals

The implants used in this study were stainless steel with the chemical composition of the metal that is shown as follows: Cr: 19.22%, Ni: 7.8%, Mn: 1.2%, Si: 0.5%, C: 0.019% and Fe: Balance, see Table 1. Samples of stainless steel for electrochemical measurements were machined down to 1 mm in diameter, 3 mm in width and approximately 6 mm in length. They were polished with different grit emery papers up to 4/0 grade, cleaned with distilled water and rinsed in ethanol before mounted in an electrochemical cell. The sample was partially immersed to a constant depth in the testing solution during the experiments. Testing solution of phosphate buffer saline (PBS) [8.77g dm^{-3} sodium chloride (NaCl), 1.42 g dm^{-3} di-sodium hydrogen phosphate (Na_2HPO_4) and 2.72 g dm^{-3} potassium di-hydrogen phosphate (KH_2PO_4)] was prepared from analytical grade reagents and triply distilled water. The test solution was adjusted at pH = 7.4. This test solution was chosen to simulate the physiological solution in order to be able to compare the *in vitro* results with the *in vivo* data.

Table 1. Chemical compositions (wt%) of the stainless steel samples before (blank) and after immersion in physiological solution (PBS).

Sample	O	Si	Fe	Mn	Ni	Cr	C	Na	P	K	Cl
Blank	-	0.50	68.13	1.22	7.82	19.67	3.11	-	-	-	-
Immersed for 14 days	16.67	-	40.17	0.77	4.34	10.87	7.31	8.19	5.41	1.33	4.94

2.2 *In vitro* experimental and analysis

Electrochemical and corrosion test

The samples were immersed in simulated solution (PBS) during 14 days. All electrochemical measurements were accomplished with an Autolab (PGSTAT30 with GPES and FRA modules, Ecochemie) in a one compartment three-electrode cell where a platinum wire counter electrode (CE) and a saturated calomel electrode (SCE) as reference to which all potentials are referred. The working electrode (WE) was in the form of a plate cut where the exposed surface areas of the investigated materials was 0.16 cm^2. The potentiodynamic current - potential curves were recorded by changing the electrode potential automatically from – 800 mV to + 2500 mV, just after exposition to the electrolyte solution. The potential scan rate was 1 mV/s. Corrosion current densities (I_{corr}) and corrosion potential (E_{corr}) were evaluated from the intersection of the linear anodic and cathodic branches of the potentiodynamic curve as Tafel plots. Electrochemical impedance spectroscopy (EIS) is a non-destructive sensitive technique which enables the detection of any changes occurring at the electrode/electrolyte interface. Impedance data were presented as Bode plots. Bode plots are recommended as standard impedance plots, since all impedance data are equally represented and the phase angle, θ, is a sensitive parameter for any surface changes. All EIS spectra were acquired by applying the open circuit potential over a frequency range of 10^{-1}–10^5 Hz to evaluate the structure stability of stainless steel in PBS. Samples were tested at two periods of immersion time. The results were analyzed using the fit program FRA (Fadl-Allah and Mohsen, 2010). Before impedance or polarization measurements, the working electrodes were immersed in the test solution until a steady state of the open-

circuit potential was reached. Each experiment was performed at least twice with a new surface for each run.

Microstructure characterization of surface

Before the polarization experiments, the scanning electron microscope (SEM) photographs were carried out for stainless steel samples to study the morphology of samples before and after immersion in the physiological solution, using SEM Model Philips XL 30 attached with EDX Unit and accelerating voltage 30 kV., magnifications from 1500X up to 15.000X. Samples were coated with a thin layer of gold to prevent charge problem and enhance the resolution. The composition of the surface film, before and after immersion of the samples in physiological solution, was characterized by EDX analysis.

2.3 *In vivo* experiments and analysis

Experimental animals and implantation

Wistar rats weighing 90-100 g (n = 10) were purchased from King Fahed Medical Research Centre in Jeddah (Kingdom of Saudi Arabia). They were acclimatized and fed *ad libitum* with rodent chow and tap water for a minimum of seven days before surgical process. Animals were randomly divided into two groups with five animals in each; control group and laminar implants group. The European Community Directive (86/609/EEC) and National rules on animal care have been followed. The animals were anesthetized intraperitoneally with a solution of 8 mg ketamine chlorlhydrate and 1.28 mg xylazine per 100 g body weight. The skin of right tibiae was shaved before a 1.5 cm incision was made along the tibial crest. The region of surgery surface was cleaned with antiseptic. The subcutaneous tissue, muscles and ligaments were dissected to expose the lateral external surface of the diaphyseal bone. An end-cutting bur was used to drill a hole 1.5 mm in diameter with manual rotating movements to avoid overheating and necrosis of the bone tissue (Cabrini et al., 1993). No cooling with NaCl was required. No antibiotic therapy was administered. Laminar implants of commercially stainless steel implants of 3.0 × 1.0 × 1.0 mm exhibited a predominantly smooth surface with irregularities that are characteristic of the lamination process. After 14 day of post-implantation, the blood samples were collected from animals in the tube containing ethylenediaminetetraacetic acids (EDTA) under light anaesthesia for blood analysis.

Haematological and Biochemical parameters

Blood samples were collected from the retro-orbital plexus vein of the animals according to Schermer. Blood samples were transferred to test tubes containing EDTA for haematological parameters [red blood cell (RBC) counts, haemoglobin (Hb), packed cells volume (PCV), white blood cell (WBC) counts, lymphocytes counts, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and thrombocytes] using Systemax KX21N haematology analyzer. Each sample was run in duplicate.

Liver was removed from rat under ether anaesthesia after 14 day of implantation and washed with cold saline buffer. Washed tissues were immediately stored at -80 ºC. To obtain the enzymatic extract, tissues were homogenized in ice cold 50 mM sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA to yield 10% (W/V) homogenate. The homogenates were then centrifuged at 4000 rpm for 10 min at 4 ºC. The supernatant were separated and used for determination of enzymes activity of alanine aminotransferase (ALT), a separate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and glutathione-S-transferase (GST). The data expressed in international units per gram (IU/g). These biomarkers for liver damage were determined using UV kinetics methodology of the commercial diagnostic kit (Stanbio Co., Spain). Total protein was determined using bovine serum albumin (BSA) as standard and values were expressed as mg/g.

The lipid peroxidation (LPO) was estimated as the concentration of thiobarbituric acid reactive product malondialdehyde (MDA) by using the method of Ohkawa et al. (1979). It was measured spectrophotometrically at 532 nm by using 1,1,3,3-tetraethoxypropane as an external standard. LPO was expressed as MDA in µmol/g of liver tissue. Glutathione (GSH) was measured in tissue homogenates of liver after reaction with 5, 5′-dithiobis-(2-nitrobenzoic acid) using the method of Beutler et al. (1969). The GSH content was expressed as mM GSH/g tissue using a calibration curve prepared by known concentrations of reduced glutathione.

Histopathology

Histopathological examination was carried out according to Drury and Wallington (1980) at the end of the experiment. The animals were killed by ether overdose; the tibiae were removed and fixed in 10% formalin solution for 14–18 h, EDTA solution is used to decalcify bone specimens for histological examination. Then, the samples passed in a series of graded ethanol and embedded in paraffin. Paraffin sections were cut at 5 µm thickness by rotatory microtome and stained with haematoxylin and eosin (H & E) stain for light microscopic examination.

Statistical analysis

Statistical analysis was based on comparing the values between laminar implants group and control group. The results are expressed as means ± SD (n=5). Statistical comparisons were performed using One-way Analysis of Variance (ANOVA) using SPSS statistical software package version 13. The level of significance was taken below $P < 0.05$.

3. Results

3.1 *In vitro* electrochemical measurements

Potentiodynamic polarization

The potentiodynamic polarization technique was used to investigate the electrochemical behaviour of stainless steel in physiological solution, PBS. Representative polarization curves from the potentiodynamic polarization measurements are displayed in Figure. 1. The quantitative corrosion values of corrosion potential (E_{corr}), corrosion current density (I_{corr}), passivation current density (I_{pass}) and pitting potential (E_{pit}) obtained through the polarization curves were calculated and are presented in Table 2. The greatest negative E_{corr} value of -300 mV was observed for the specimen just immersed in PBS. The E_{corr} of specimen was shifted in the noble direction with the time of immersion in PBS. Hysteresis through potential ranging from -300 to 600 mV was found for all stainless steel samples in PBS. These results refer to the passivity is not stable, and indicative of pitting corrosion.

Table 2. Electrochemical parameters calculated from the potentiodynamic polarization curves at different times of immersion of stainless steel samples in physiological solution (PBS).

Immersion time	E_{corr} / mV_{SCE}	E_{pitt} / mV_{SCE}	I_{corr} / A cm^{-2}	I_{pass} / A cm^{-2}
Zero time	-300	300	8×10^{-7}	6×10^{-2}
1 day	-20	650	2×10^{-8}	4×10^{-2}
14 days	-250	800	2×10^{-8}	2×10^{-3}

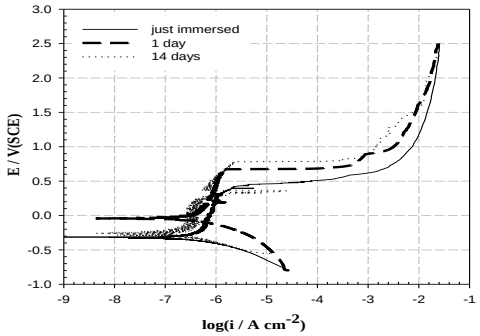


Figure. 1**.** Polarization curves obtained for Stainless Steel after different times of immersion in phosphate buffer saline (PBS) at pH 7.4.

Impedance measurements

The impedance results for the stainless steel in physiological solution after immersion for two different times are shown in Figure 2. These spectra indicate that the long immersion time of samples in PBS plays an important role to change the properties of the passive film formed on samples. The Bode plots recorded for the stainless steel specimens after zero time (just immersed) and two weeks of immersion in PBS are presented in Figure 2a and 2b respectively. The impedance bode plot (Figure 2a) show that, the impedance resistance of the specimen change with the time of immersion. It is noted that, the impedance values at the minimum frequency range, which correspond to the corrosion resistance of the electrode material, increases with increases the time of specimen immersion in PBS. On the other hand, the phase bode plot (Figure 2b) show that only one phase maximum, which suggests that the time constant of the protective film RC circuit is much greater than that of the double layer RC circuit. This result refer to the corrosion process was mainly charge transfer controlled. The general shape of the curves is similar for all the stainless steel specimens, indicating that almost no change in the corrosion mechanism occurred due to the immersion time (Rosliza et al., 2008). Another parameters related to impedance analysis derived by curve fitting method are summarized in Table 3. Generally, the equivalent circuit model is representing the surface properties of stainless steel specimen in PBS solution and it considers a good way that can be proposed to simulate the experimental results appropriately. Figures 2a and 2b show the computer fitted values and experimental impedance data of specimen in PBS. Charge transfer resistance R_{ct} and capacitance C were obtained by fitting the spectra (0.1-1000Hz) using a simple equivalent mode (Figure 2c). The results showed that R_{ct} values increased with increasing immersion time, the capacitance, C values decrease indicating the formation of a surface film.

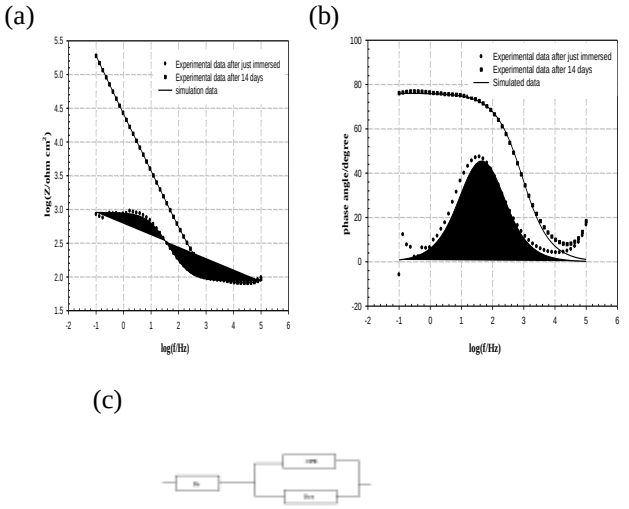


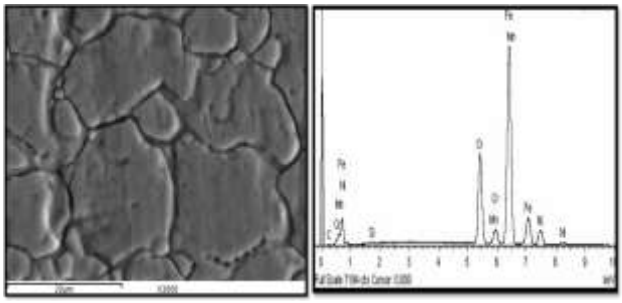
Figure. 2. Impedance data recorded for stainless steel at different immersion times in phosphat buffer saline (PBS) at pH 7.4. (a) Bode-impedance pots, (b) Bode-phase plots, (c) Equivalent circuit used for fitting experimental impedance data where R_s is the solution resistance, R_{ct} is the charge transfer resistance, and CPE is a constant phase elements.

Morphology and composition analysis

Figure 3a presents the SEM of the mechanically polished stainless steel surface. It is observed that the microstructure is characterized by irregularly shaped intermetallic grains with different sizes and their surface distribution is not very homogeneous. This figure indicates that the surface of the specimen is one of the coarse surfaces which increase the biocompatibility of the metal. The grains are separated from each other by grain boundaries contain small grooves and neither precipitates nor bulk impurities were observed. Figure 3b shows the EDX photographs of the specimen of Figure 3a. Figure 4a shows the SEM of the stainless steel specimen immersed after the aforementioned periods of immersion in PBS. The SEM of Figure 4a shows that the specimen surface after immersion in PBS is covered with a thin slightly homogeneous film. The SEM of high magnification demonstrates the presence of relatively less number of holes between the covered a thin film, Figure 4b. The major observation zoom up more where it was noted the disappearance of the majority of these holes (Figure 4c). Figure 4d shows the EDX photograph of the immersed specimen after 14 days in PBS where there were typical high resolution spectra for Fe and Cr. The concentration of Fe and Cr decreased after exposures as compared to the sample before exposure as indicated in Table 1.

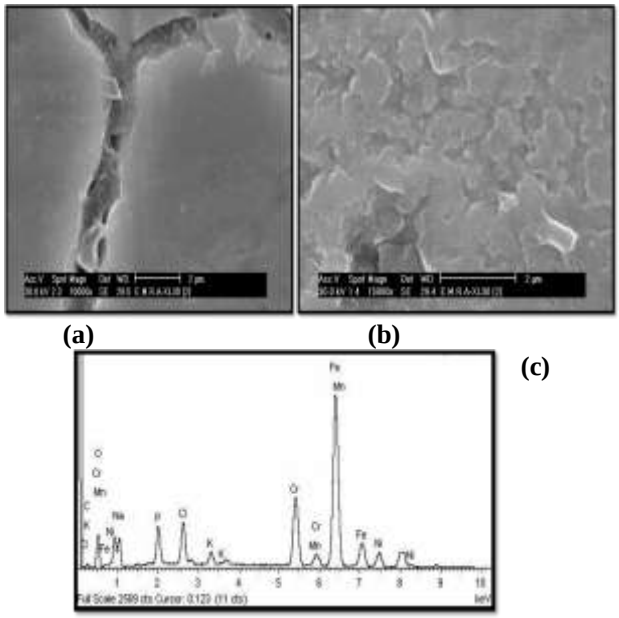
Table 3. Polarization resistance values are calculated by EIS measurements at different time of immersion of stainless steel samples in physiological solution (PBS).

Immersion time	R_s / Ω	R_{ct} / KΩ cm^2	*CPE*/μF cm^{-2}	n
Zero time	84.51	0.9352	27.74	0.858
14 day	108.29	12491	7.93	0.896



(a) (b)

Figure. 3 (a) Scanning electron micrograph, SEM, and (b) EDX photographs of stainless steel before immersion in phosphate buffer saline (PBS).



(a) **(b)** **(c)**

Figure 4. (a) Scanning electron micrograph of stainless steel after immersion for 14 days in phosphate buffer saline (PBS) at pH 7.4 with magnification 1500 X, (b) with magnification 10000 X, (c) with magnification 15000 X, and (d) EDX analysis.

3.2 *In vivo* investigation

After implantation of the stainless steel laminar no rat displayed inflammation and there were no unexpected deaths. No weight reduction in any animal was seen during the experimental period. Good healing around the implants was observed without any dehiscence or inflammation.

Table 4. Effect of Stainless Steel implantation for 14 days on haematological parameters of male rat

Parameter	Experimental group	
	Control group	Implanted group
RBCs Parameters		
Hb (g/dl)	9.93 ± 0.47	10.80 ± 0.25
PCV (%)	30.63 ± 3.81	33.33 ± 3.84
TEC (X 10^6)	5.98 ± 0.04	6.15 ± 0.07
RBCs indices		
MCV (fl)	42.40 ± 0.10	42.90 ± 0.01
MCH (pg)	15.60 ± 0.02	17.00 ± 0.12
MCHC (%)	27.20 ± 1.10	25.20 ± 0.34
TLC and DLC %		
TLC (X 10^3)	10.30 ± 0.45	7.00 ± 0.06 [a]
L (%)	69.67 ± 1.20	56.00 ± 0.58 [a]
N (%)	31.67 ± 0.88	44.00 ± 0.58 [a]
Platelets (10^3/mm^3)	667.33 ± 2.73	561.67 ± 13.39 [a]

Hematological and biochemical assay

Hb concentration, PCV% and RBC count slightly increased in the implanted-group. No statistically significant changes were detected in MCH, MCV and MCHC values in implanted-animals as compared to control group at the end of study (Table 4). A significant decrease ($P < 0.01$) in total leukocyte count (TLC) specially percentage of lymphocytes was noticed in implanted-group as compared to the control group, while there was a significant increase ($P < 0.003$) in neutrophils count, reaching to 38.9% of the control value. The significant decrease ($P < 0.05$) in the activities of AST, ALT ALP and LDH (Table 5) after operation could be expected to occur associated with pathology involving necrosis of the liver. As shown in Table 5 the hepatic enzymes LDH and ALP significantly decreased by 2.1-fold and 4.5-fold, respectively, in the implanted-rats as compared to control group indicating plasma membrane damage and liver injury. Antioxidant and oxidative stress of stainless steel corrosion was evaluated by measuring hepatic GST, GSH and LPO (Table 5 and Figure 5) of rat after 14 day of implantation. The present results showed a significant increase in hepatic LPO in stainless steel-implanted rats (Fig. 5). In the present study, decreasing of GSH (Fig. 5) levels in liver tissue under short-term implantation is considered as the primary response to an oxidative-stress-inducing stainless steel.

Values expressed as mean ± SE of 5 separate animals in each group. Blood haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total leukocyte count (TLC), differentiation of leukocytes (DLC), lymphocytes (L), neutrophils (N) and platelets count. [a]Significant different as implanted group compared to control group ($P < 0.001$).

Increasing LPO was associated with the alterations in GST activity (Figure 5 and Table 5). There was an increase in the GST activity at the end of two weeks of stainless steel-implantation ($P < 0.05$) (Table 5).

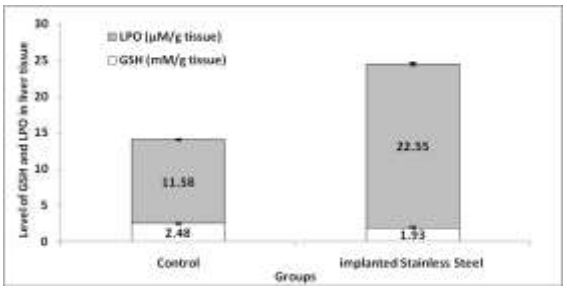


Figure 5. Glutathione (GSH) and lipid peroxidation (LPO) levels of hepatic tissue of male rat after 2 weeks of stainless steel implantation.

New bone formation after two weeks of post-implantation as well as a gap between the residual implant and the surrounding bone tissues can be observed in Figure 6A-6D. There are newly formed trabecular and osteoblasts (Figure 6B), indicating that the hole in the tibia gradually healed during degradation of the stainless steel.

Bone tissue histology

The early inflammatory phase comprises the first 2 weeks post injury and is initiated after haemorrhage caused by vascular injury and the subsequent development of a hematoma (Figure 6A and 6B). Infiltration of inflammatory cells and of fibroblasts into the area then occurs (Figure 6A). These events lead to vascularisation of the area and the formation of granulation tissue (that is, procallus) (Figure 6B). The repair phase is characterized by the formation of a callus. It begins with continued vascular in growth, secretion of osteoid, and the presence of fibro collagenous fibers. A temporary callus consisting of cartilage is produced at the site of injury (Figure 6C). Osteoblasts continue to be active and replace cartilage with the cancellous bone forming a bridge between the fractured fragments (Figure 6D).

4. Discussion

4.*1 In vitro* study

The potentiodynamic polarization technique was used to investigate the electrochemical behaviour of stainless steel in PBS. Table 2 shows that the Ecorr for the specimen after 1day of immersion in PBS become more positive than that for the specimen just immersed. This observation suggests that the passive layer is readily formed on stainless steel when it is just immersed in physiological solution. Although, the values of Ecorr for the stainless steel specimen immersed for two weeks is shifted to positive values than that just immersed (Figure.1), it is noted that this Ecorr value is slightly lower than that of stainless specimen immersed for 1day in PBS. This observation probably refers to slightly affected of protection layer with the passage of time for stainless steel specimen in PBS.

As can be seen in Figure 1, it was not possible to evaluate the cathodic Tafel slope as there is no visible linear region that prevents linear extrapolation to E_{corr} of the cathodic polarization curves. This irregularity was confirmed by other researchers and can be explained as the superposition of at least two cathodic current contributions: one arises from oxygen reduction and the second one consequential of metal ion re-deposition (Khaled et al., 2009). It was possible in this case to evaluate, I_{corr}, by extrapolation of the anodic polarization curves only to E_{corr}.

The appearance of hysteresis in the potential region from -300 to 600 mV is evidence that passivity is not stable, and indicative of pitting corrosion. The occurrence of these oscillations was explained by the consecutive formation and repassivation of micro size pits, indicating the formation of so-called metastable pits. The metastable pits are very small in size and grow and repassivate in less than few seconds (Mohammed, 2009). The increase in the current density after hysteresis region is considered an indication of may be complete transition of metastable to stable pitting occurs at E_{pit}. It is noted that the stainless steel immersed for longer time in PBS exhibited more positive E_{pit} values at room temperature than specimens immersed for zero time or short time (Table 2). Although, E_{pitt} is shifted to more positive values when the time of stainless steel immersion increases to two weeks, huge hysteresis is observed. This observation could explain the strong competitive adsorption of cations present in PBS, as Na, K, P, with chloride ions at active surface sits. This explanation was considered as the reason of good corrosion resistance of stainless steel in PBS especially after long period of time in physiological solution (Zuo et al., 2002). The polarization behaviour of all stainless steel specimens in PBS was observed to be almost similar by occurrence of an order of magnitude lower I_{corr} (Table 2). The present study showed that the presence of alloying elements play a significant role in the development of protective film on stainless steel. Generally, this result suggests that this type of stainless steel is quite *in vitro* effective in simulated physiological solution through the formation of a thin protective layer. This explanation is supported by the SEM and EDX analysis.

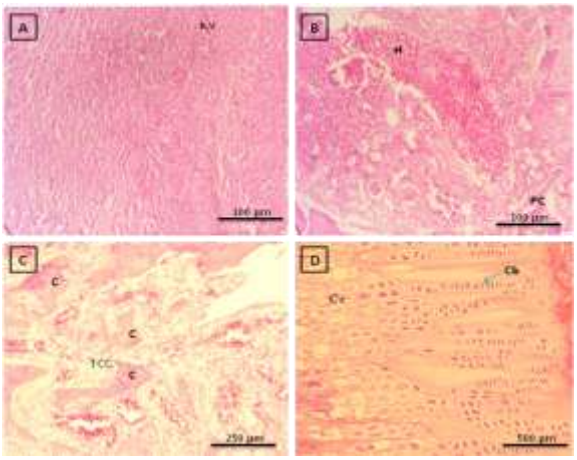


Figure. 6. Photomicrographs H&E stained tissues two weeks after implantation. *A:* Early inflammatory phase of fracture healing is characterized by organizing hematoma. This photomicrograph demonstrates in growth of inflammatory cells, fibroblasts, and small blood vessels (B.V.) into a blood clot, B: The reparative phase consists of fibrosis and woven bone production, characterized by irregular trabeculae of immature bone and osteoid rimmed by osteoblasts, as well as reactive fibro vascular stroma, hematoma (H) and procallaus (PC), C: The late reparative phase is characterized clinically by hard callus formation and temporary cartilage cell, D: Reactive cartilage that is undergoing endochondral ossification; cartilage is growing (expanding) toward the left and cartilage with hypertrophying chondrocytes (Cc) (at left) however, the condroblast (Cb) at right.

The Experimental impedance data were fitted to theoretical data according to different equivalent circuits representing the electrode /electrolyte interface. The best fit was obtained using the simple equivalent circuit presented in Figure 2c. Figure 2a and 2b show the computer fitted values and experimental impedance data of the stainless steel specimens in PBS. This model consists of a parallel combination of a resistor, R_{ct}, representing the charge transfer (corrosion) resistance, and a capacitor, C, representing the electrode capacitance, in series with a resistor, R_s representing the ohmic drop in the electrolyte. However, better fitting is obtained when constant phase elements (CPE), is used in place of pure capacitance, which it is associated to rough surfaces (Fadl-allah and Mohsen, 2010). The CPE can also include a contribution from dynamic disorder such as diffusion (Al-Mobarak et al., 2006). There is a good agreement between the experimental and theoretical data according to the proposed model. The deviation observed in Figure 2a and 2b can be attributed to the fact that the protective film formed after zero immersion is less homogeneous than that formed after 14 days immersion in PBS.

Impedance parameters derived by curve fitting method are summarized in Table 3. The results showed that R_{ct} values increased with increasing immersion time, the pseudo- capacitive elements present in the circuit are in fact constant phase elements, CPE values decrease indicating the formation of a surface film. The long time of immersion increase the corrosion resistance from 1KΩ to 12491KΩ, but they have of the same order of magnitude (KΩ).The enhancement in corrosion resistance of specimen after long time of immersion can be attributed to formation of passive film can prevent aggressive ions from strong attacking the substrate (Chaves et al., 2006). These findings are consistent with the results obtained by the polarization potentiodynamic tests which observed in Figure 1.Therefore, we can be expect that the formed film after 14 day of specimen immersion in PSB has corrosion resistance up to a good degree but still limited. However, it is also susceptible for the diffusion of chloride ions from PBS through its film/metal interfaces.

It was important to confirm the electrochemical investigations by structural and compositional investigations. Therefore, SEM and EDX measurements were carried out. Many researchers have focused on the imaging the surface of specimen after electrochemical polarization measurements (Elki et al., 2010), but we focused in this study on the imaging metal after enough time of immersion in the simulated physiological solution.

The presence of such a slightly homogeneous film is also confirmed by the EIS characteristics. The observation of morphology and composition analysis supported the results from the potentiodynamic polarization, where we note initiation and passivation of pitting. The presence of these ions (Na, K, P, etc) is confirmed by the EDX investigations, which indicates that the incorporation of the ions from the physiological solution to the protective film and lead to increase its corrosion resistance by time. Moreover, Ni was found after exposure for 14 days with small amount, which suggest that Ni was more completely dissolved from the specimen to the solution during the exposure (Marcus et al., 2008). On the other hand, Cl^- was detected by EDX analysis, which indicates that Cl^- is involved in the surface reaction. This observation supports the results from the polarization and EIS measurements. The penetrations of the chloride ions to the material surface initiate holes which accumulate to form the corrosion spots shown in this scanning electron micrograph.

4.2 *In vivo* study

Enhancement of HB and number of RBCs could be evidence that anemia is not associated with degradation of stainless steel during implantation. Decreasing the TLC could be due to excessive production of wear particles from stainless steel. Rena et al. (2008) reported that TLC plays an important role in engulf particulate debris and becomes activated; releasing proinflammatory cytokines, chemokines, degradative enzymes and reactive oxygen radicals. A significant decrease in platelets count was detected in implanted-group indicated that the rats had thrombocytopenia.

The leakage of intracellular enzymes serves as an index of liver injury, as this enzyme is present in large quantities in the liver (Dufuor et al., 2000; McCuskey and Sipes, 2002). These enzymes escape to the plasma from the injured hepatic cells when cellular degeneration or destruction occurs in this organ (Dufuor et al., 2000). The degradation of the stainless steel as proved *in vitro* study (Table 1) could be the reason to decrease the hepatic enzymes activity after its implantation in rat. The heavy metals in the stainless steel allow derogated by 41.0, 36.88, 44.5 and 44.74% for Fe, Mn, Ni and Cr, respectively after 14 day in PSB. The decrease in the activities of ALP in different tissues might be due to the increased permeability of plasma membrane or cellular necrosis (McCuskey and Sipes, 2002), and this showing the stress condition in implanted-animals.

Decrease in hepatic proteins referred to liver dysfunction as proven by the decrease in liver enzymes activities. The *in vitro* study showed that corrosion products released into the surrounding tissues by stainless 316L orthopaedic implants may affect the expression of the osteogenic phenotype; the *in vivo* mice model, used to investigate the systemic effects induced by the corrosion products *per se*, shed some light on the possible consequences of metal accumulation in organs of vital importance such as the liver, the kidney and the spleen **(**Morals et al., 2000). Mattson (1998) reported that Cr released from implant alloy (polymethylmethacrylate cemented cobalt-chromium) and increased in serum after implantation of the alloy.

Due to high concentration of polyunsaturated fatty acids in cells, LPO is a major outcome of the free radical mediated injury. Two broad outcomes of LPO are structural damage of cellular membranes and generation of oxidized products, some of which are chemically reactive and may covalently modify cellular macromolecules. These reactive products are thought to be the major effecter of tissue damage from LPO (Mattson, 1998). LPO is the additional an indicator of hepatic oxidative injury. This suggests participation of free-radicals induced oxidative cell injury in mediating the toxicity of stainless steel. It was also found that LPO induced by aluminum at sub-lethal levels, alter physiological and biochemical characteristics of biological systems. LPO indicated that there is an unbalance between the productions of oxidants and scavenging of those oxidants by antioxidants (Jaeschke et al., 2002).The result was confirmed by measuring the levels of hepatic GSH. Reduced GSH possesses antioxidant properties and its protective role against oxidative-stress-induced toxicity by keeping reduced of the proteins' –SH groups.

The ability to generate ROS and oxidant injury is one paradigm that may be used to compare the toxic potential of nanoparticles (Xia et al., 2006). Oxidative stress is a state of redox disequilibrium in which ROS production (by the cell or by the nanomaterial itself) overwhelms the antioxidant defense capacity of the cell, thereby leading to adverse biological consequences (Xia et al., 2006): damage of macromolecules, lipids, DNA or proteins resulting in excess cell proliferation, apoptosis, lipid peroxidation, or mutagenesis. The ROS formation by redox reactions may be a critical event in toxic effects if the protective mechanisms of cells are overwhelmed by a strong affinity with nanoparticles. Metallic nanoparticles are known to induce oxidative stress by ROS generation during redox cycling by disruption of the electronic and ionic flux, perturbation of the permeability transition pores and depletion of the cellular glutathione content (Xia et al., 2006).

GST catalyse the addition of the tripeptide glutathione to endogenous and xenobiotics substrates which have electrophilic functional groups. The glutathione adducts produced have increased solubility in water and are subsequently enzymatically degraded to mercapturates and excreted (Hayes et al., 2005). This result confirmed by decreasing the GSH level in implanted-rats which scavenges residual free radicals escaping decomposition by the antioxidant enzymes. GSH plays an excellent role in protecting the cell from LPO.

Oliveira et al. (2004) reported that the effect of metal mixtures (Cu^{2+}+Zn^{2+}, Zn^{2+}+Fe^{2+}, Zn^{2+}+Cr (VI), and Cr (VI)+Fe^{2+}) (100 µM) on liver microsomal EROD activity was assessed, revealing a synergistic interaction. Therefore, the toxicity of stainless steel in implanted rat could be related to the biodegradation of the alloy and releasing Fe, Mn, Ni and Cr in the rat tissue as indicated *in vitro* study.

There are three main phases following fracture in the bone repair process: 1) the early inflammatory stage; 2) the repair stage; and 3) the remodelling stage. This observations have been described by Hansen-Algenstaedt et al. (2006**)** during the bone repair, These events lead to vascularisation of the area and the formation of granulation tissue (that is, procallus). This initial union has limited strength; thus, internal/external immobilization of the fracture/fusion site is often appropriate. In the remodeling phase the process may occur over months to years and consists of restoring the fractured bone to its normal size, shape, and strength. There was histological evidence of successful bone regeneration, at 2 weeks new bone covered pore space and there was scarce thin fibrous band at the surface near the stainless steels implants.

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A Review of the Problems Faced by AIOU Regional Centers in Pakistan

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Abstract: The objective of the study was to investigate the problems faced by the regional centers of Allama Iqbal Open University (AIOU) Pakistan. For the purpose of collection of data, a questionnaire was developed and the data collected through the questionnaire were tabulated, analyzed, and interpreted. Major findings of the study reveal that the major problems faced by AIOU regional centers staff are the limited frequency of capacity building workshops, shortage of transport facility, and the absence of purpose-built infrastructures for the regional centers. Overcoming these deficiencies at the regional centers will result in better working environment at these centers and hence yield to overall better performance.

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1. Introduction

In distance education system, the teaching is decentralized and learners are given instruction and guidance preferably where they live or at the nearest place. According to Perraton (1978), distance teaching means an educational process in which significant proportion of teaching is conducted by someone moved in space and or time from the learner" while Rehman (1998) has given the following definition of distance education: Education conducted by the postal services without face-to-face contact between teacher and learner. Teaching is done by written or tape-recorded materials sent to the learner, whose progress is monitored through written or taped exercises to the teacher, who corrects them and returns them to the learner with criticism and advice. The above definition of distance education, adopted by UNESCO, is very simple and indeed a realistic one. Actually, the student in distance education is an individual who cannot attend classes and does not have the luxury of coming face-to-face with the teacher in a classroom environment, at least for extended period of time. A student always needs guidance and assessment of his performance w i t h regard to his academic ability and achievement. It is provided by a teacher/tutor who guides and helps from a distance. Harris and William (1977) state that the term “distance training” includes "any planned or regular educational provision where there is a distance between teacher (or instructor or educator) on the one hand and student (or learner or receptive audience) on the other hand."

Need and Nature of Distance Education

Distance education plays complimentary, supplementary, as well as an independent role. It provides cost-effective education to diversified target groups eliminating the class disparity. David (1983) has discussed the need and nature of distance education in these words:

The distance education whether concerned with elementary, university, occupational or professional study regularly includes three types of activities on the part of the organization that administers it. First of all, the development of self-instructional study material or courses printed and/or recorded which may either be self-contained or of a study guide type relying on set text. Secondly, teaching at distance by comments in writing, on the telephone or an audiocassette on student work submitted. Thirdly, general support and counseling of student work by the same distance study media.

Allama Iqbal Open University (AIOU) has been a pioneer in providing distance education to the wide masses of Pakistan population for years. It has facilitated easy access to education for working class men and women. In case of women, its performance is even more admirable keeping in the view the traditional culture of Pakistan. Faure (1972) is of the view that:

Due to unequal growth rate, the educational needs and demands are both increasing to a great extent in the whole world. Such growing needs are producing pressure to institutions and resulting in out of school education. So much of national resources are being allocated to education. AIOU, being a distance education institution, relies heavily on all varieties of available media to reach its students in appropriate manner.

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According to the University's Vice-Chancellor Annual Report (AIOU (2004)) the main components of AIOU's distance education package are the following:

- Correspondence materials: including self-learning study package and supplementary study materials, such as textbooks and study guides, delivered by mail.
- Radio and television broadcasts: generally related to the study materials of the package. AIOU has been airing educational media material on PTV-2 television channel which is beamed through satellite to more than 45 countries.
- Online Teaching: Various regional centers of AIOU are being linked for online education through teleconferencing, CDs, and other recorded media are being provided to the students.
- Non-broadcast media; including slides, audio/video cassettes, flip charts, and leaflets, commonly for basic functional and literacy level courses.
- Tutorial instruction: as face-to-face contact sessions, practical labwork sessions, and academic guidance sessions at regional study centers. For postgraduate programs such as M.A., M.Sc., M. Phil, Ph.D., group training workshops are conducted while short-term/long-term internships in industrial or business concerns for BBA and MBA programs are a mandatory component of these programs. The students are given course assignments which are evaluated by the tutors and final examinations are held for each course at the end of the semester.

Thus, distance education system of AIOU ranges from the most traditional methodology to highly advanced satellite and internet media. This wide range provides easy learning opportunities to students belonging to various areas of Pakistan.

Government Policy and Plans for Distance Education

Allana (1990) has discussed the growing need of distance education in Pakistan in this manner:

There is vast scope for Allama Iqbal Open University to start different types of both formal and non-formal education programs. The following relatively recent developments are indicators of current planning. Though the Allama Iqbal Open University offers its courses to both male and female populations without any discrimination, it has established a Department of Women's Education which takes exclusive care of specialized courses for women. In addition to home economics, food and nutrition, etc. it has started a project for providing education at matriculation level for out-of-school women, which could be extended to the whole of Pakistan in a phased manner and then converted into a regular program of the Allama Iqbal Open University. Further, the Department of Literacy, Adult and Continuing Education is also embarking upon an Integrated Functional Education Program for the 10+ age group of females. Since the participation rate of females at primary and subsequent stages is already very low, there is enormous scope for launching courses designed for women's education in the near future. The advent of modern information technology, the use of satellites and, most importantly, the internet have opened new vistas in the field of distance education. As the access to internet increases for the general population, the University will be in a position to supplement its instructional efforts through the internet throughout Pakistan. The traditional system of correspondence, tutorials, and written words w ill be gradually replaced by on-line teaching through the internet. Hussain (2002) has discussed the setting of future policy for Open and Distance Learning (ODL) in Pakistan through AIOU as the following:

The University is trying to get a license for starting its own full time Radio and Television Stations to harness them in support of its educational programs. Similarly, the University has already made a significant beginning in the fields of Basic and Applied Sciences. Science and Technology is the focus of academic expansion in the next five years or so. The University has made a beginning in the field of medicine. It has launched post graduate diplomas in eye care, nutrition and dietetics with the expertise of recognized hospital in the country. A1OU has taken on a special role in the remote northern areas. The National Education Policy 1998-2010 also expects radio and television to play crucial role and be extensively used for social mobilization and promoting the cause of basic education, particularly amongst rural females, and to impart life skills to the new literates.

Problems in Distance Education System

Jumani (2003) and Saleem (1987) have pointed out the problems faced by distance education system as the following:

- Firstly, in distance education system, printed material is dispatched to the students by post at their postal addresses. The postal services

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are slow and unreliable in Pakistan. Sometimes this material is dispatched to a wrong person or the students may get their mail late.

- Secondly, there is a shortage of trained staff for managing distance education, particularly staff like writers, course designers, coordinators, reviewers, etc. to prepare distance education material.
- Thirdly, in any education system, evaluation is essential i.e. internal and external assessment. Internal assessment, which includes students' assignments, is not working up to the mark. Sometimes students fail to submit their assignments or tutors do not evaluate the assignments and this leads to many problems.

Furthermore, Saleem (1987) has pointed out that expansion of distance education into science and engineering programs is not easy in developing countries due to difficulties in the development of appropriate instructional material, availability of well equipped laboratories, high expenses of use of electronic media, inadequate cooperation from conventional universities and research laboratories, and high cost involved in setting up own laboratories at different places.

Similarly, the usefulness and relevance of AIOU radio and TV programs is also not certain. The large student audience has diverse needs and problems. Different students have different problems. These radio and TV programs may not be effective for such divergent needs and problems of the students. Moreover, students' problems cannot be addressed at the spot that makes students lose interest in studies. Similarly, reaching out to the students, especially women, in far-flung villages is very difficult and expensive, at least at the initial stage. Thus, the cost of education may be higher than conventional education. This creates another hurdle in the way of the students to get education through distance education system.

Sometimes even the credibility of distance education degree or certificates is at stake due to wrong perception of people who think that degree or certificate obtained through distance education is not of the same standard as the degree obtained through the conventional face-to-face educational system.

Critics of distance education say that this mode of education does not socialize its students up to the required standards due to infrequent face to face contact whereas it is the distinctive characteristic of conventional university. Distance education in developed as well as in developing countries faces numerous challenges. The equivalence, social recognition, media, information, material, mailing, trained faculty etc are problems and issues pertaining to distance education all over the world. Jenkins (1993) has observed that:

Growth in distance education has been very fast, and it is not easy to identify the areas in which success is most significant. Judging by the extent of provision, programs in management have done remarkably well. No doubt this is partly due to demand, but continuing and growing demand is, in itself, a measure of success. When one considers the extent to which management training involves interpersonal interaction, discussion and practical application, its success in the distance mode, which can be weak in precisely these areas, is of great interest. A study of why distance education in this area is proving so effective appears to be overdue. Another area which needs further study is costs. As distance education first became popular, it was often sold to governments on the argument of its cost effectiveness. More learners could be taught more cheaply than by conventional means, it is now recognized that this argument could be misleading. Though cost effectiveness is of critical importance, it is not necessarily achieved by large numbers. The issue is by no means simple. Quality may sometimes suffer if costs are cut too far, large schemes are not always what is needed, reaching marginal students may be essential though costly. The issue of the costs borne by students is often overlooked.

In view of the above discussion, it is clear that distance education has its benefits as well as problems which need to be addressed to make it an effective educational system.

Establishment of Allama Iqbal Open University

Informal distance education programs were initiated in Pakistan during the 1960s by the Pakistan Television Corporation and the Pakistan Broadcasting Corporation. The formal establishment of the first distance education institution in Pakistan was by the name of Peoples Open University, which was later renamed as Allama Iqbal Open University. It would not be wrong to say that Allama Iqbal Open University education system is based on scientific and modern teaching practices employed across the world. The University utilizes latest multimedia approach and multidimensional methodologies to make teaching and learning process simple, easy, and interesting.

Objectives of Allama Iqbal Open University

According to AIOU Act (1974) the main objectives of the University are described as the following:

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- To provide facilities to the masses for their education in such manner it may determine.
- To provide facilities for the training of teachers in such manner it may determine.
- To provide for instruction in such branches of technology or vocation, and to make provision for research and for the advancement and dissemination of knowledge in such manner it may determine.
- To hold examinations and to award and confer degrees, diplomas, certificates and other academic distinctions.

Institutional Framework of Allama Iqbal Open University

The distance education system followed by Allama Iqbal Open University has proven its potential for expansion and growth. Hussain (2002) has stated that: "(AIOU)… has supplemented the efforts of both the federal and provincial governments by easing their load, and served the citizens of the nation by making educational access more available." In this respect its largest contribution has been in making education accessible to the female learners and the working people. According to AIOU Act (1974), AIOU was set up with the following framework:

There shall be established a University to be called the Peoples Open University as a specialized educational institution with its Principal seat at Islamabad for purposes of teaching, holding of examinations and tests, establishment of audience level regional centers for guidance and examinations at the post-matric, graduate, post-graduate and research levels in accordance with the provision of this Act. The Peoples Open University was renamed as the Allama Iqbal Open University by an (amendment) Ordinance 1977. The University shall consist of:

The Chancellor, the Pro-Chancellor, the Vice-Chancellor, the Deans, the Chairmen of Teaching Departments, Directors of the Institutes, the Director of the Regional Tutorial Services, the Registrar, the Treasurer, the Auditor, the Controller of Examinations, the Librarian, and such other officers as may be prescribed.

- Members of the Executive Council, the Academic Council and other Authorities.
- All University teachers; and
- Professors Emeritus.

Organization and Administration of Allama Iqbal Open University

The Vice Chancellor in his Annual Report (2004) has explained the administrative structure of Allama Iqbal Open University as the following:

The organizational structure of the University is based on the three areas of academic, service and administrative departments. AIOU is meeting and managing the vast educational needs through its four faculties i.e. Faculty of Arabic and Islamic Studies, Faculty of Sciences, Faculty of Social Sciences and Humanities, Faculty of Education, and Institute of Mass Education. The collective academic strength at the main campus in Islamabad is 200, with 734 supporting staff. Total staff is 364 and total number of course offerings is over 850. The statutory bodies of the university include the following:

- Executive council
- Academic Council
- Board of Advanced Studies and Research
- Academic Planning and Development
- Research and Educational Technology Committee
- Faculty Board of each faculty
- Committee of Courses
- Selection Board
- Finance Committee
- Administrative Departments

In addition to the faculties consisting of academic departments, there are also several other administrative, supervisory, and service departments working under the Vice- Chancellor. These departments support the academic needs of the Allama Iqbal Open University system. According to AIOU in Brief (2005), the following are the administrative departments at Allama Iqbal Open University:

- Registrar's Department
- Treasurer's Department
- Audit Department
- Project Directorate
- Public Relations Office

Service Departments

According to AIOU in Brief (2005), the following are the administrative departments at Allama Iqbal Open University:

- Admissions
- Examinations
- Bureau of Academic Planning and Course Production
- Institute of Educational Technology

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- Print Production Unit
- Editing Cell
- Directorate of Regional Services
- Students Advisory and Counseling Cell
- Central Library
- Computer Center
- Research and Evaluation Center

Regional System Network

The outreach system of the University in the form of its Regional Campuses and Centers is the backbone of its instructional methodology. The regional campuses/centers play an essential role in distance education. The head of a region prepares rosters of experts in the region in all the subject areas. He/She arranges qualified tutors from amongst these experts for each group of students. The students have the opportunity to meet the tutors at least twice a month except in case of low enrollment courses for which correspondence tutors are appointed. The head of the region also arranges collaboration with relevant local institutions and registers them as Study Centers. These centers organize workshops/practical training in the regions. Examination centers are also identified and arranged by the regional head. Recently all the regions have been linked with the central database through e-mail/fax/internet to facilitate better and faster communication, removal of complaints, and to enable decentralization as much as possible. Besides this, there are 101 part-time regional coordinating offices in different parts of the country for providing assistance to the regional campuses/centers. There are more than 60,000 part-time registered tutors with the University for tutorial support and guidance.

According to Vice Chancellor's Annual Report (AIOU (2004)) the University has the following 36 regional campuses/centers in the country:

Punjab
1. Regional Campus Rawalpindi
2. Regional Campus Lahore
3. Regional Campus Faisalabad
4. Regional Campus Multan
5. Regional Center Gujranwala
6. Regional Center Bahawalpur
7. Regional Center Sahiwal
8. Regional Center Sargodha
9. Regional Center Mianwali
10. Regional Center D.G.Khan
11. Regional Center Jhang
12. Regional Center Sialkot
13. Regional Center Chakwal
14. Regional Center Rahim Yar Khan

Sindh
15. Regional Campus Karachi
16. Regional Campus Hyderabad
17. Regional Center Sukkur
18. Regional Center Mithi
19. Regional Center Dadu
20. Regional Center Thatta
21. Regional Center Larkana

Khyber Pakhtunkhuwa
22. Regional Campus Peshawar
23. Regional Center Abbottabad
24. Regional Center Dera Ismail Khan
25. Regional Center Saidu Sharif
26. Regional Center Chitral

Balochistan
27. Regional Campus Quetta
28. Regional Center Zhob
29. Regional Center Dera Murad Jamali
30. Regional Center Turbat
31. Regional Center Kalat

AJK/Northern Areas
32. Regional Campus Mirpur
33. Regional Center Muzaffarabad
34. Regional Center Gilgit
35. Regional Center Skardu

Islamabad Capital Territory
36. Regional Center Islamabad

2. The Aim of the Current Research

The current research was designed to determine the problems faced by the regional campuses/centers of the Allama Iqbal Open University.

3. Population

All the heads of Regional Campuses/Centers (BPS-17 and above) constituted the population of the study.

4. Sample

100% heads of Regional Campuses/Centers were taken as sample for the study.

5. Instrument

One questionnaire (closed and open ended) was prepared and discussed with experts of the field to validate it with respect to content, language and format. It was improved in the light of their suggestions. The questionnaire was also pilot-tested using officers other than the heads not included in the sample. Necessary improvements were made to make a final version of the questionnaire.

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6. Collection of Data

The questionnaire was sent to the regional campuses/centers through registered mail along with a self-addressed stamped envelope for reply. Request for quick response was made through telephone and e-mail. Responses from all the heads of the regions were received.

7. Analysis of Data

The collected data were tabulated and analyzed by applying mean score. The data collected through open-ended questions were analyzed by using priority frequency of responses. The following formula was used to calculate the mean score:

Mean score = .X/N
Where .X = Sum of the scores
N = Total number of responses

The data obtained were tabulated in term of frequency. The frequencies were converted into score by assigning the following scale value to each of the five responses:

SA	= Strongly Agree	05 points
A	= Agree	04 points
UNC	= Uncertain	03 points
DA	= Disagree	02 points
SDA	= Strongly Disagree	01 points

Table 1: Results of Data Analysis

Statement	SA	A	UNC	DA	SDA	Mean score
The facilities by Allama Iqbal Open University lo the Regional campuses/center are up-to the mark.	7	20	1	4	0	3.94
	84%		3%	13%		
Regional campus/center is provided required funds for providing satisfactory services, especially under the following heads: (i) Postage (ii) Telephone (iii) POL (iv) Stationery	6	26	0	0	0	4.19
	100%		0%	0%		
Regional campus/center is provided required funds for providing satisfactory services, especially adequate instructional material is provided to the Regional campus/center	3	18	6	3	2	3.53
	65%		19%	16%		
Adequate transport is available	5	12	4	8	3	3.25
	54%		12%	34%		
Communications are responded to within reasonable period.	8	22	2	0	0	4.19
	94%		6%	0%		
Electronic devices such as Fax & Internet is available at Regional campus/center.	8	14	0	0	0	4.56
	100%		0%	0%		
DRS has a direct and regular contact with the regions.	24	8	0	0	0	4.75
	100%		0%	0%		
Problems/difficulties of regions are solved within reasonable time.	13	19	0	0	0	4.41
	100%		0%	0%		
DRS has a system of monitoring the working of the regions.	16	13	3	0	0	4.41
	91%		9%	0%		
Update information is communicated to the Regions.	13	19		0	0	
	100%		0%	0%		
Capacity building workshops for the offices in the regions are conducted by DRS/University Administration.	7	6	7	10	2	3.19
	41%		22%	37%		
Queries are responded to in time.	4	22	6	0	0	3.94
	81%		19%	0%		
Meeting of the Regional Heads are arranged for interaction between the personnel of the Regions and Main Campus.	10	17	5	0	0	4.14
	84%		16	0%		
Financial claims of the office are cleared quickly.	2	24	4	2	0	3.81
	81%		13%	6%		
Furniture that the meet the needs of the Region.	11	17	4	0	0	4.22
	87%		13%	0%		
Building facility is enough to meet the needs of the Regions.	3	15	8	6	0	3.28
	56%		25%	19%		

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8. Summary of the Findings

Following is a summary of the findings from the data analysis:

- 84% of the respondents agreed with the statement that the facilities provided by AIOU to the regional campuses/centers were up to the mark.
- 100% of the respondents agreed with the statement that regional campuses/centers were provided requisite funds for postage, telephone, POL and stationery.
- Majority of the respondents i.e. 65% agreed that adequate instructional materials were provided to the regional campuses/centers.
- It was observed that 54% of the respondents agreed that adequate transport was available.
- Majority of the respondent i.e. 94% of the respondents agreed that communications were responded to within reasonable period.
- 100% of respondents agreed that electronic communication facilities, such as fax and internet, were available at regional campuses/centers.
- All the respondents agreed that DRS has a direct and regular contact with the regions.
- All of the respondents agreed that problems/difficulties of regions were solved within reasonable time.
- 91% respondents agreed that DRS have a system of monitoring the working of the regions.
- All of the respondents agreed that up-to-date information was communicated to the regions.
- 41% of the respondents agreed that capacity building workshops in the regions are conducted by the University.
- Majority of the respondents i.e. 81% agreed that queries were responded to in time.
- 84% respondents agreed that meetings with the Regional Heads were arranged for interaction between the personnel of the regions and the main campus.
- 81% of the respondents agreed that financial claims of the offices were cleared quickly.
- 87% of the respondents agreed that existing furniture meets the needs of the region concerned.
- It was found that 56% of the respondents agreed that building infrastructure was enough to meet the needs of the region.

9. Conclusions

After analyzing the result of the survey, it is clearly evident that, based on majority positive responses on the whole, all regional campuses/centers of AIOU are satisfied with the facilities provided to them to impart distance education to the students in their specific regions. Starting from physical infrastructure to electronic communication facilities, provision of postage and stationery to regular contact with the DRS and AIOU administration, provision of adequate transport facilities to holding of capacity building workshops, in short, in every aspect, regional campuses/centers are able to cope with the responsibility of distance education in Pakistan in an efficient and admirable manner.

10. Recommendations for enhancing AIOU distance education experience

Following is a summary of the recommendations made by regional campuses/centers for enhancement of AIOU distance education experience in Pakistan:

- Building infrastructures always have a vital role in the improvement of organizational performance. It is recommended that AIOU should build purpose-built infrastructure for every regional center with the latest modern
- Distance education facilities like video conferencing and the internet etc. In addition, there should be a facility for boarding and lodging of students and visitors at the regional centers.
- In-service training/ refresher courses enhance the capability and performance of the officers/employees of any institution. Therefore, frequency of capacity building workshops/trainings in the regions may be increased by the University.
- Provision of adequate means of transport is an essential need of students as well as staff of AIOU regional campuses/centers. Currently only 54% of the regional centers agree that this facility is adequately provided to them. Efforts need to be made to enhance the provision and quality of this service to the regional centers. Administrative problems either become stumbling blocks or add to the stress of the employees and, as a result, an organization fails to achieve its objectives efficiently and effectively. If neglected for a long time they become root cause of conflicts, underperformance, disillusion, and low quality. They also block creativity in the long run and thus deprive the organization from the ability to evolve and grow. Therefore, it is vitally important that AIOU administration should take initiatives to implement the

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recommendations mentioned above so that the regional centers can provide best possible services to the students for quality education.

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Comparison between Outer Membrane Protein Profile of Fluoroquinolones Sensitive and Resistant *P. aeruginosa* Isolated from Egyptian Patients

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Abstract: *Pseudomonas aeruginosa* is an important opportunistic pathogen that infects immunocompromised hosts and is characterized by its natural resistance to a variety of antimicrobial agents. The purpose of this study was the assessment of the fluoroquinolones resistance level among *P. aeruginosa* clinical isolates, furthermore to compare between the outer membrane protein profile of fluoroquinolones susceptible and resistant isolates of *P. aeruginosa* using Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) technique. Sixty five (43%) were identified as *P. aeruginosa* by conventional culture techniques. MIC of ciprofloxacin, norfloxacin and levofloxacin against pseudomonal isolates were determined by twofold agar dilution technique. Only about 39%, 40% and 42% of these isolates were resistant to ciprofloxacin, levofloxacin and norfloxacin, respectively. Profile of outer membrane protein fraction of the fluoroquinolones resistant isolates showed an additional band with an approximate molecular weight of 50-54 kDa. In conclusion, overproduction of outer membrane protein of approximate molecular weight 50-54 kDa in *P. aeruginosa* was associated with fluoroquinolones resistance.

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Keywords: *Pseudomonas aeruginosa*- fluoroquinolones- outer membrane protein- SDS_PAGE

1. Introduction:

Pseudomonas aeruginosa is a clinically significant opportunistic pathogen that infects immunocompromised hospitalized patients and is characterized by its innate resistance to a variety of antimicrobial agents. Only a few antimicrobial agents, such as quinolones, show potent antibacterial activity against this species (Zeng, 2004; Pitt and Simpson, 2006). In recent years, a number of clinical *P. aeruginosa* isolates were reported to be resistant to the quinolones, because of the presence of an outer membrane with a low level of permeability and thereby intrinsically resistant to a wide variety of commonly used antibiotics (Lambert, 2002). The quinolones class of antibiotics generally has retained excellent in vitro activity against many common Gram-negative bacterial pathogens (Sárközy, 2001; Scholar, 2003; Van Bambeke *et al.*, 2005). However, as these agents are more frequently prescribed, increasing rates of bacterial resistance are being reported (Hooper, 2001; Ruiz, 2003). Several mutations conferring quinolones resistance have been identified and mapped on *P. aeruginosa* chromosomes. The mutation of *gyrA* (*nfxA*, *nalA*, or *cfxA*) causes an alteration in the subunit A of DNA gyrase (Hsu *et al.*, 2005; Niga *et al.*, 2005), while the *nalB* (*cfxB*) (Li and Poole, 2001; Hocquet *et al.*, 2004), *nfxB* (Chuanchuen *et al.*, 2005; Jeannot *et al.*, 2008), and *nfxC* (Fukuda *et al.*, 1995) mutations decrease the level of quinolones accumulation, and strains with the later mutations show cross-resistance to structurally unrelated antimicrobial agents. The present study aimed to compare between the outer membrane protein profiles of fluoroquinolones susceptible and resistant isolates of *P. aeruginosa.*

2. Materials and methods

Antibiotics:

Ciprofloxacin hydrochloride monohydrate and norfloxacin were provided as a gift from E.I.P.I.CO. Pharmaceutical Company (10th of Ramadan City, Egypt), while levofloxacin hemihydrate standard powder was provided from MUP Pharmaceutical company (Ismailia, Egypt).

Bacterial isolates:

One-hundred and fifty bacterial isolates were isolated from cases admitted to Educational Suez Canal University Hospital, Ismailia, Egypt. The isolates were identified using the conventional culture and biochemical techniques (Cheesbrough, 2000; Goldman and Green, 2009).

Susceptibility testing:

MIC_S were determined by the usual twofold agar dilution technique with Mueller–Hinton agar (*Difco,USA*) and an inoculum size of 10^4 cells according to the guidelines of standard procedures (Schwalbe *et al.*, 2007; CLSI, 2007).

Assay of outer membrane proteins:

The method of Masuda *et al.* was followed, briefly resistant isolates were isolated by plating on Mueller–Hinton agar (*Difco,USA*) plates containing sub-MIC of each fluoroquinolone. The sub-MICs ranged from 2-4, 8-16 or 4-8 µg/ml for ciprofloxacin, norfloxacin and levofloxacin, respectively. The largest colony in each plate was picked up and recultivated in Mueller–Hinton broth (MHB; Difco, USA) containing the same concentrations of antibiotics, in which they grew. While sensitive isolates were cultivated directly in antibiotic- free Mueller–Hinton broth (MHB; Difco, USA). The overnight growing cells in MHB were harvested by centrifugation at 12300×g for 5 min at room temperature. Harvested cells were suspended in 0.1M Tris-HCl; pH 8.0. Suspended cells were broken with a sonicator for 3 min. Unbroken cells were removed by centrifugation at 4000×g for 10 min at room temperature. Membranes were pelleted by ultracentrifugation at 38000×g for 1 h at 6^0C. The inner membrane was solubilized by adding 2% sodium N-lauroylsarcosinate (sarkosel) in 10mM HEBES buffer (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) pH 8 to the pellets, this was followed by overnight incubation at room temperature. The outer membrane was pelleted by centrifugation at 18,000×g for 40 min at 6^0C and resuspended in 0.1M Tris-HCL buffer; pH 8.

Analysis of outer membrane protein:

The outer membrane fraction was analyzed by SDS-PAGE as reported by Sambrook *et al.*, with 12.5 % polyacrylamide resolving gel and a 5% stacking gel. The analyzed samples were treated with reducing sample buffer, pH 6.8 that containing 0.2% SDS and 2% β-mercaptoethanol. Treated samples were immersed in boiling water bath for 5 min, and then they were subjected to electrophoresis at a constant current of 200 mA at room temperature. The gel was stained with silver staining using silver staining kit *(Pharmacia Biotech).*

3. Results and Discussion:

Sixty- five out of one-hundred and fifty (43%) clinical bacterial isolates were identified as *P. aeruginosa*. The sources and numbers of the isolates which grew *P. aeruginosa* were burn – 37; urine – 15 and wound — 13. Results showed that out of the 65 *P. aeruginosa* isolates 25 (39%), 26 (40%) and 27 (42%) isolates were resistant to ciprofloxacin, levofloxacin and norfloxacin, respectively (Table1).

In agreement with Swiatlo *et al.* (2000), Oliphant and Green (2002) and Emami *et al.* (2005) the present study showed that cross-resistance existed between these fluoroquinolones.

Electrophoresis of outer membrane protein (OMP) fraction on SDS-PAGE gel revealed that fluoroquinolones- resistant isolates differed in OMP profile from the susceptible ones. An additional protein band with an approximate molecular mass of 50-54 kDa appeared in each of ciprofloxacin-resistant isolates (lane 2), norfloxacin-resistant isolates (lane 3), and levofloxacin-resistant isolates (lane 4) when compared with sensitive isolates (lanes 5, 6 and 7) (Figure 1).

These results suggested that the outer membrane protein may be involved in *P. aeruginosa* resistance to fluoroquinolones, in agreement with Masuda *et al.* (1995); Alonso *et al.* (1999); Le Thomas *et al.* (2001) and Griffith *et al.* (2006).

The previous was agreed with Le Thomas *et al.* (2001) and Nakajima *et al.* (2002); when they used probing technique for probing the outer membrane of fluoroquinolones-resistant mutant with outer membrane protein-specific antibody, they demonstrated a 2-3 fold overexpression of an outer membrane protein named, OprM. This evidence agreed with Masuda and Ohya (1992), who suggested that overproduction of OprM is associated with resistance to fluoroquinolones in *P. aeruginosa*.

These reports agreed with Köhler *et al.* (1999), Poole (2000) and Griffith *et al.* (2006) who suggested that, the elevated intrinsic resistance in *P. aeruginosa* due to the low outer membrane permeability correlated to the appearance of 50-54kDa outer membrane proteins.

4. Conclusion

From all the previous, we can conclude that fluoroquinolones resistance in *P. aeruginosa* was associated with overproduction of 50-54 kDa outer membrane protein. This protein may be responsible for decreased drug accumulation inside the cells; making antibiotics inefficient in infected sites, and could be responsible for increasing the level of intrinsic resistance, enhancing acquired resistance, and increasing frequency of emergence of *P. aeruginosa* strains highly resistant to fluoroquinolones in clinical settings especially when combined with mutations in the target enzymes (DNA gyrase and topoisomerase IV).

Table 1: Resistant patterns of 65 *Pseudomonas aeruginosa* clinical isolates to the three fluoroquinolones.

Fluoroquinolones	NO. of Sensitive Isolates	NO. of Resistant Isolates	% of Resistance
Ciprofloxacin	40	25	39%
Norfloxacin	38	27	42%
Levofloxacin	39	26	40%

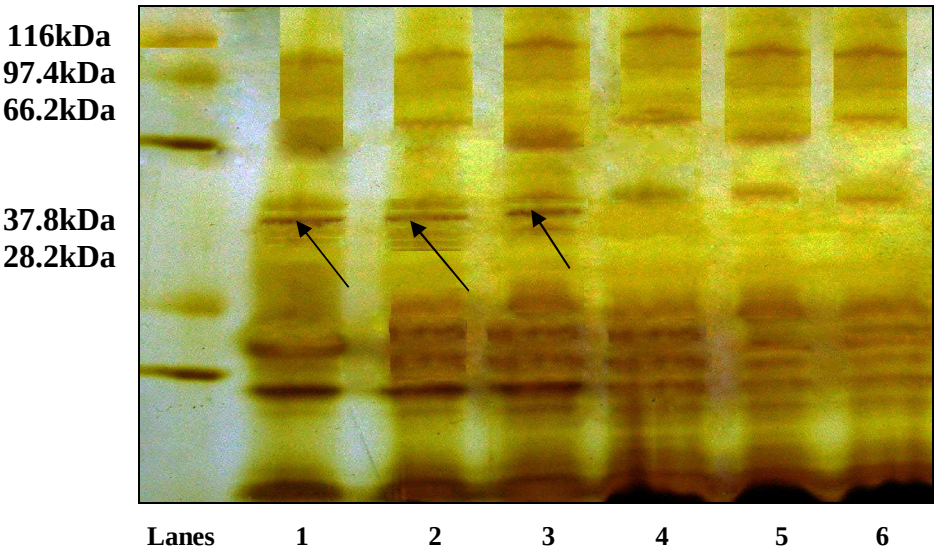

Fig. 1: Silver-stained SDS-PAGE of Sarkosel-insoluble membrane fractions: showing the outer membrane protein profile of ciprofloxacin-resistant isolates (lane: 2), norfloxacin-resistant isolates (lane: 3), and levofloxacin-resistant isolates (lane: 4), When grew in the presence of ciprofloxacin, norfloxacin or levofloxacin, respectively, these lanes obtained an outer membrane protein having electrophoretic mobility corresponding to an apparent molecular mass of approximate 50-54 kDa, arrows showing these bands. Lanes: 5, 6, & 7, showing the outer membrane protein profiles of the fluoroquinolones–susceptible isolates. Lane: 1, showing molecular weights of protein marker components (Jena Bioscience,Germany) in kDa.

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In-vivo and *in-vitro* Prediction of the Efficiency of Nano-Synthesized Material in Removal of Lead Nitrate Toxicity

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Abstract: Due to large grain sizes, the biological properties of the conventional hydroxyapatite (HAp) is limited to a great extent. Progresses in nanotechnological approaches now allow the fabrication of nano-HAp. In this study, firstly, the characters of nano-hydroxyapatite gel was described and the interaction performance of the formed gel with lead nitrate $Pb(NO_3)_2$ *in vitro* was identified. Then, the biological efficiency of nano-HAp gel against $Pb(NO_3)_2$ toxicity *in vivo* was introduced. A polymeric matrix route was selected to synthesis nano- composite hydroxyapatite gel. The formed gel characterized using FTIR, XRD, SEM, TEM. Various volumes of the produced nano-HAp gel (10, 20, 30, 40, 50 and 60 µl) was adding to 4 ml of ECS solution. The clear supernatant was separated and analyzed by ICP-MS. The results showed a successful removal of lead ions by formed gel. A single dose of intravenous nano-hydroxyapatite at a level of 150 and 300 mg/kg b.w. was injected to male rats following intraperitoneal 93mg/kg b.w. (LD_{50}) of lead nitrate $Pb(NO_3)_2$. The results revealed that nano- HAp composite had the ability to alleviate lead nitrate toxicity, to a great extent, in serum antioxidant status, liver and kidney function as well as corticosterone and calcium levels but phosphorus value was not affected among the all treated groups. However, most successful results were attributed to the treatment with high dose of formed nano-HAp particularly after 48 h more than the treatment with low dose. Histopathological observations confirmed the biochemical results, since nano-HAp into rats evident the recovery of lead nitrate cytotoxicity in liver and kidney cells.

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Keywords: Nano-HAp, lead nitrate, antioxidant status, liver enzymes, kidney functions , corticosterone

1. Introduction:

The advent of nanoscience gives human a new perspective in biology; all biological systems have their most basic structures, properties and functions defined at the nanoscale from their first level of organization and are governed by the molecular behavior at nanometer scales (Christenson *et al*. 2007). These mysterious structures and functions of the nanoscale living organelles (e.g., ribosome) and non-living nanostructures (e.g., tooth) formed within living organisms have always attracted attention and fascination. With the development of molecular and nanoscale technique and engineering, the methodologies provide tools and platforms for the creation and manipulation of complex structures and functions on the scale of nanometers (Xu *et al*. 2007). At the same time, biology also serves as the source of inspiration for creating new devices and systems integrated from the nanoscale. Thus, the fabricated nano biomaterials with biomimetic structures and functions are expected to bear high bioactivities and unexpected biological effects, and ultimately can well serve as biomedicinal devices for human disease treatment. Many commercial substitute materials now have been developed, including natural and synthetic polymers, human bones, synthetic ceramics and composites (LeGeros,2008) especially hydroxyapatite (Descamps *et al*. 2008). The reason for the development of the HAp based biomaterials is their similarity in composition to the bone mineral, and has good biocompatibility, bioactivity, osteoconductivity (Bauer *et al*. 2008 and Abel-Gawad & Awwad, 2010).

Because lead has a major health hazard throughout the world due to its disruption effect on biological systems (Sharma *et al*. 2010) and considering as carcinogenic agent (Silbergeld, 2003), it is important to explore the possibility of minimizing its effects on the body. The currently approved treatment for lead intoxication is to give chelating agents but, these chelators are potentially toxic (Flora *et al*. 2007) and often fail to remove lead from all body tissues (Sharma *et al*. 2010). For this, several methods have been investigated for the removal of lead ions using calcium phosphates specially hydroxyapatite structure (Ciobanu *et al*. 2000 and Xu *et al*. 2007). Great researches on HAp have been carried out to understand the immobilization mechanism of heavy metals from aqueous solutions and to evaluate its usage for environmental remediation (Mavropoulos *et al*. 2002). Modification of the HAp surface is a technique available for developing catalysts and adsorbents with novel functions. However, HAp is

usually provided in powder or calcined pellets form, which might be a disadvantage to recover this material after removing heavy metal ions from wastewater (Janga *et al*. 2008).

In light of the long history of therapeutic application of HAp, it hypothesized that are of this compound may be of interest in the management of heavy metal-induced disease. On the other hand, progresses in nanotechnological approaches now allow the fabrication of nanocrystalline HAp and could conceive the biological properties of nano-HAp are able to be improved evidently. Hence, in the present study, nano- composite HAp gel was prepared and characterized, then it was investigated its efficiency in removal of lead ions from aqueous solution and finally evaluated the biological effects of intravenous nano-HAp against lead toxicity in male rats including biochemical and pathological investigations.

2. Materials and methods

Chemicals:

The chemicals used for nano-HAp preparation were calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$, Mwt. 236.15 g/mole, Merk, Germany), diammonium hydrogen ortho phosphate anhydrous ($(NH_4)_2HPO_4$, 132.06g/mole, S.D. Fine Chem. Ltd. Mumbai, India), poly vinyl alcohol (PVAL) (Mwt. ≥ 160000 g/mole), and ammonium hydroxide (NH_4OH, Mwt. 35.5g/mole, May & Baker, England). Environmental calibration standard (ECS) containing 300 ppb of Pb element, Agilent, USA). The range of the elements to be tested was within a concentration of ppb depending on their natural environmental presence. All chemicals were used in the experimental work without further purification. Also, commercial kits were used for biochemical analyses as mentioned below in materials and methods section.

In vitro experimental design:

Nano-HAp composite gel was synthesis according to technique of Abdelfattah et al., (2006) and characterized using FTIR, TEM and XRD to confirm the synthesis of hydroxyapatite structure. The formed nano composite HAp gel was used for removal of lead ions from environmental calibration standard solution ECS contained 300 ppb of lead ions. The effect of nano-HAp gel amount on the removal of lead ions from ECS solution was followed by adding various volumes of the produced nano-HAp gel (10, 20, 30, 40, 50 and 60 µl) to 4 ml of ECS solution. The clear supernatant was separated and analyzed by ICP-MS. The effect of time was studied by preparing seven vials, each vial contained 4 ml of ECS solution then the optimum amount of the gel (50 µl) was added to each vial. The supernatant was decanted at different times (10, 15, 20, 25, 30, 40 45 and 50 min) and analyzed by ICP-MS. The supernatant solution was decanted and analyzed by ICP-MS at constant time interval and the results were recorded. The formed nano gel ion mixture were analyzed using TEM and FTIR.

In vivo experimental design:

Animals and treatments:

Adult healthy male rats (140-160 g) were allowed to acclimate at the animal facilities for two weeks before use. They were housed under standard environmental conditions with free access food and water throughout the experiment. The rats were divided into four groups, control and three treated groups (each of 15 rats). Each of the treated groups received intravenous (through tail vein) single dose (93 mg/kg b.w., LD_{50}) of $Pb(NO_3)_2$ according to (ATSDR, 1993). Following three hours, the third and fourth groups received single dose of intravenous nano hydeoxyapatite (HAp) at a dose of 150 and 300 mg/kg b.w., respectively (Abel-Gawad and Awwad, 2010). Saline solution was administered to control group in the same manner as in the treated groups as a solvent for lead nitrate and nano-HAp. Blood samples were collected from orbital venous plexus at a time intervals of 3 and 48 hours for all biochemical parameters except calcium and phosphorus were estimated at times intervals 3, 24 and 48 h after nano-HAp injection. The blood centrifuged at 3000 r.p.m. and obtained serum was stored at -20ºC till analyses through a week. The abdomen of all rats were dissected immediately to remove livers and kidneys and stored in 10% formalin solution for histopathological examination.

Biochemical investigation:

Obtained serum was analyzed for the following biochemical parameters: superoxide dismutase (SOD), catalase (CAT) and glutathion peroxidase (GPx) activity were measured using commercial kits purchased from (BioVesion Research Products, USA) according to McCord & Fridovich (1988), Chelikani *et al.* (2004) and Ran *et al.* (2007) technique, respectively. Malondialdehyde (MDA) was determined (Northwest, Life Science Specialties,LLC, USA) by using Nair *et al.* (2008) method. Arginase (Gentaur Comp., Europe), gamma glutamyle transferase (GGT) (Shenzhen Mindray Bio-Medical Electronics Co.Ltd, Germany), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Right Choice Diagnostics, Ltd, Germany) were analyzed according to the methods of Crombez & Cederbaum (2005), Thomas, (1998) and Young (1990), respectively. Also, kidney function was estimated through the evaluation of urea

and creatinine levels in serum by using (Vitro Scient. Diagnostics, Egypt) according to the method of Tietz, (1990) and Tegar-Nelsson (1961). Calcium and phosphorus levels were determined using kits purchased from (Biotron Diagnostics, INC., USA) according to Woo and Cannon (1984) and Young (1990) techniques, respectively. Corticoesterone value was estimated using solid phase RIA technique according to Saino *et al*. (1988).

Histopathological investigation:

Small pieces of liver 3-5 mm thick were fixed in 10% formalin solution for 24 h. They were washed in running water for 24 h. They were then dehydrated by passing through ascending grades of alcohol: 50, 70, 90 and 100% for 2-3 days following which they were cleared in benzene to remove alcohol till the tissues became more or less transparent. They were later passed through three cups containing molten paraffin at 58C and finally embedded in a cubical block of paraffin made by the L moulds.

From the embedded samples, sections of 6 microns thick were cut using the microtone and fixed on a slide by Mayer's albumen. The sections were stained with hematoxylin to cover the section and kept for 6 min. Excess stain was removed with tap water. Eosin was added to cover the stem for 2 min. Excess eosin was poured away and removed with tap water. This was covered with a cover glass to avoid air bubbles, and viewed with the use of both low and high power microscope (Banchroft *et al*., 1996).

Statistical Analysis:

All values were expressed as mean ± SE. Statistical analysis was performed with two way analysis of variance (ANOVA) followed by Duncan's *t* ' test. *P* values < 0.05 were considered to be statistically significant.

3. Results

In vitro experimental results:

Characterization of the formed gel:

The XRD analysis of the dried powder at 100°C for 24 h identified the presence of nano-HAp crystal structure. The characteristic XRD pattern of nano-HAp (main peak d=2.81 Å and secondary peaks at d=2.78 Å, d= 2.72 Å) still exist (Fig. 1) with a small shift due to the presence of lead nitrate. It is to be noticed that the presence of lead nitrate didn't change the crystal structure of nano-HAp. The decrease of the peaks intensity is mainly due to reduced content of the nano-HAp concentration, and confirmed with IR spectrum which proved the formation of nano-HAp structure with no any other calcium phosphate structure. The microstructure analysis by SEM/EDAX of 0.1 M lead nitrate with nano-HAp gel dried at 100^{o} for 24 h was shown a presence of lead and nitrogen while the Ca/P ratio is approved the presence of nano-HAp crystals structure (Fig. 2).

The FTIR analysis of the formed HAp-gel without drying and nano-HAp–gel with lead nitrate (Fig. 3) were shown the of OH^{-1}at 3570 cm^{-1} has disappeared while the of OH^{-1} at 630 cm^{-1} has decreased. The broadening of characteristic bands of PO_4^{3-} at 1091 and 1036 cm^{-1} compared to the sharp beak of HAp could be due to the decrease of the crystallinity of HAp by the presence of lead ions. Due to the gel content in the OH band The FTIR analysis of the formed gel shows a brooded OH band due to gel content.

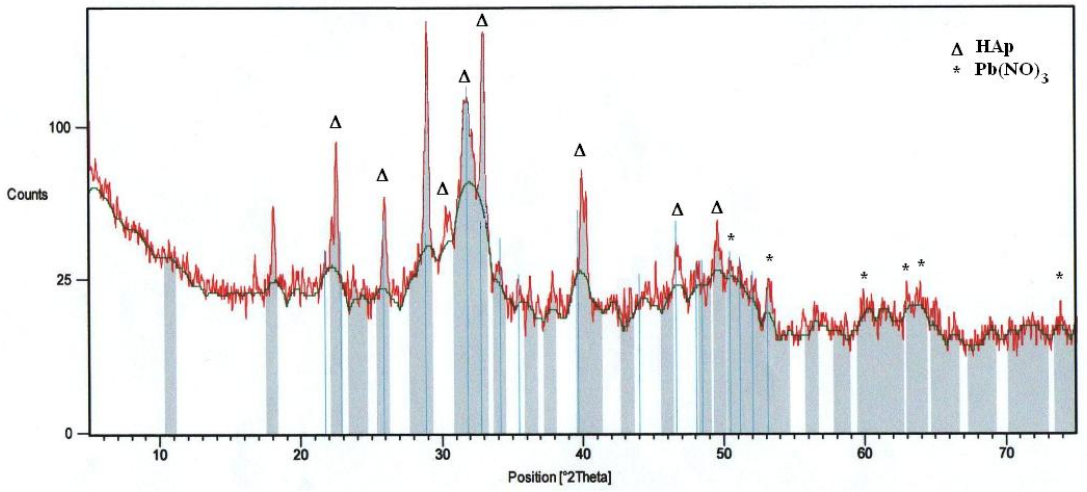


Fig.1 : XRD patterns of the nano-HAp gel with lead nitrate dried at 100°C for 24 h

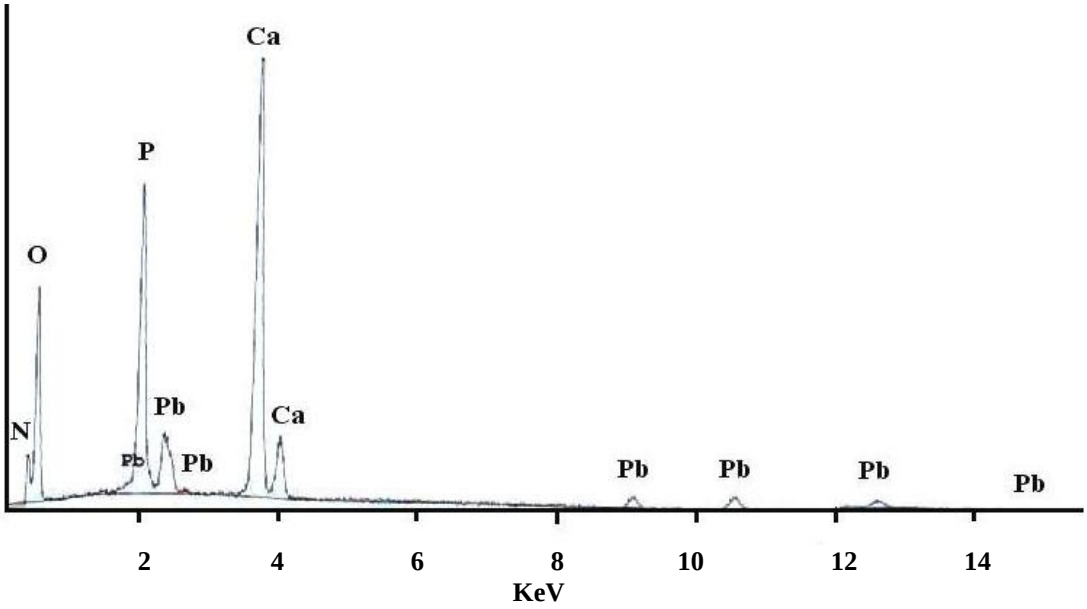


Fig.2 : EDAX analysis of 0.1M lead nitrate with nano-HAp gel dried at 100°C for 24h

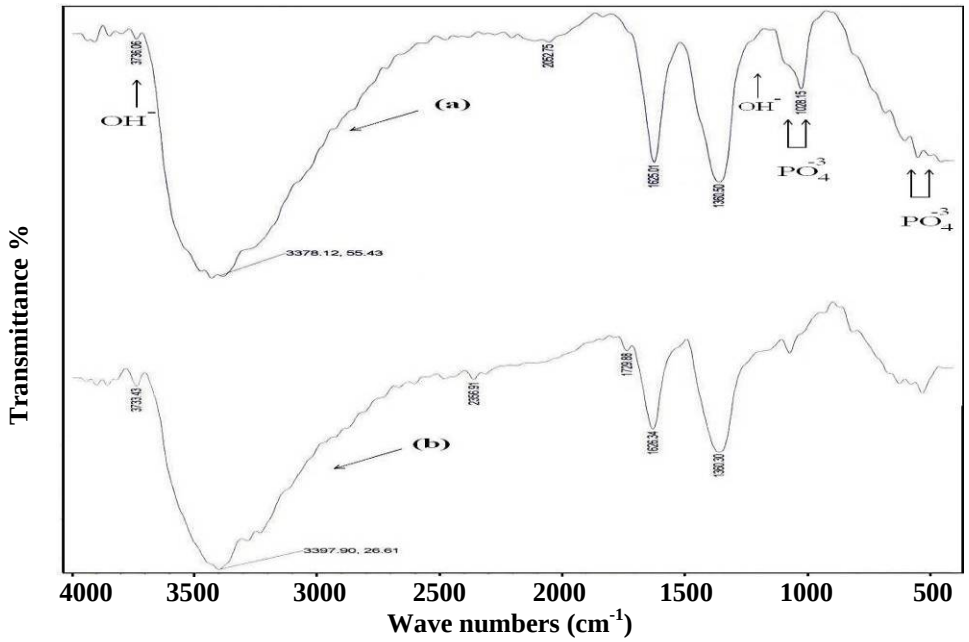


Fig. 3: IR spectra of a) nano-HAp-gel and b) nano-HAp gel with lead nitrate.

The transmission electron microscope (TEM) showed a nano-HAp of ultra small crystals distributed in matrix as shown in figure 4 with average grain size of 40 nm. TEM micrograph depicted the precipitation of hydroxyapatite aggregates in porous poly (vinyl-alcohol)–gelatin matrix. TEM studies showed a uniform distributed of nano-HAp with self-assembled and aggregates of uniform size and morphology.

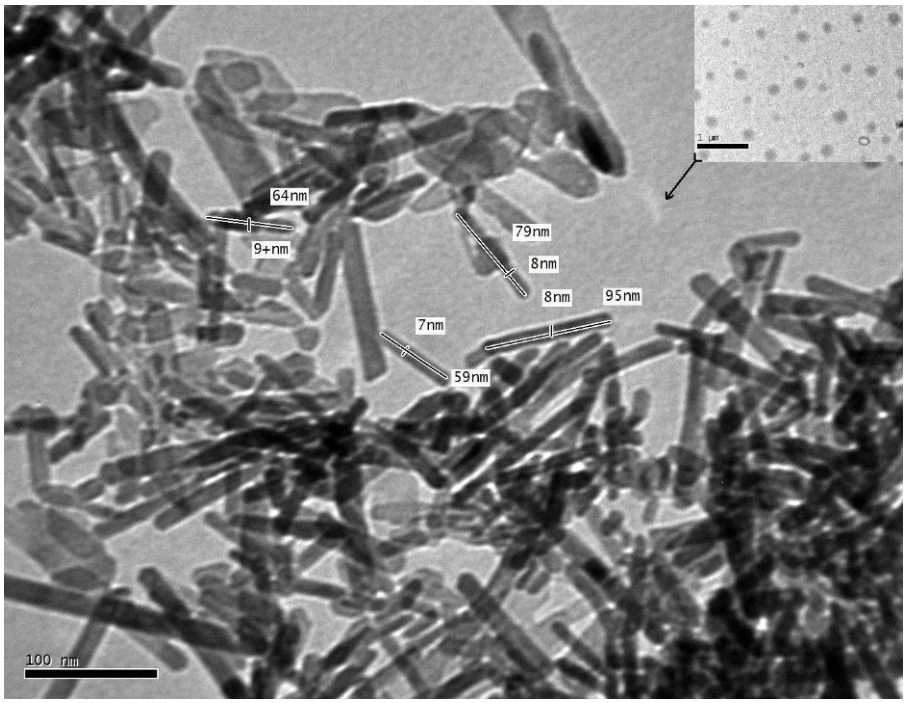


Fig.4: TEM micrograph analysis of nano-HAp gel and lead nitrate trapped in its polymeric matrix.

Due to the very fine size of precipitated particles present in the aggregates, they could not be individually resolved, however, their crystalline nature and phase identification could be ascertained through selected area diffraction pattern which confirm the formation of nano-hydroxyapatite phase. While the TEM with the nano-HAp gel with lead nitrate was shown the lead nitrated trapped in the gel polymeric matrix with average size about 72nm(Fig. 4).

Removal of lead ions:

The ICP-MS analysis of the lead ions in ECS solution contains 300ppb of lead ions were shown a slightly increase in calcium ions while the lead ions remain around control value 300 ppb(Fig. 5). While the analysis of supernatant solution of ECS solution contains 300ppb lead ions and 50 µL of nano-HAp gel was shown a decreased in lead ions gradually by time and complete indictable after 45 min (Fig. 6).

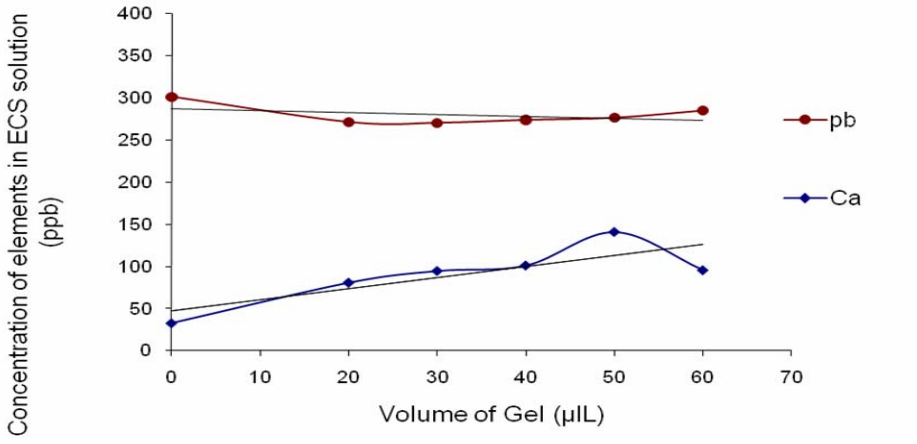


Fig. 5: The effect of adding different volumes of the formed gel to the ECS solution contains lead ions.

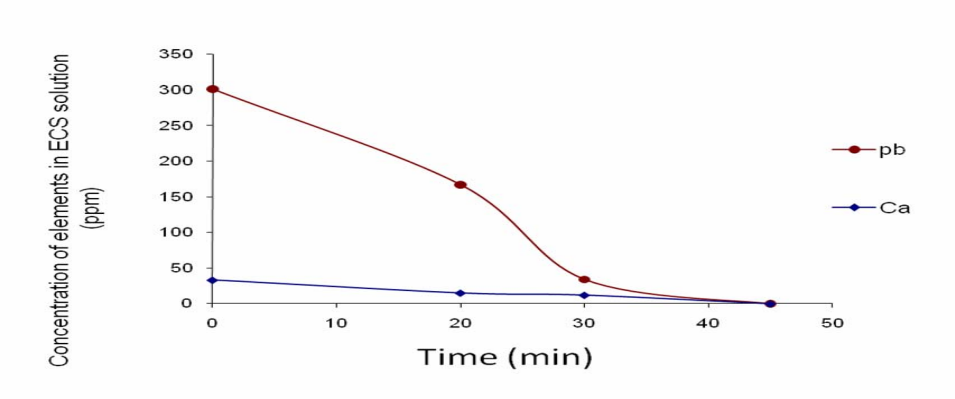


Fig. 6: The lead and calcium ions analysis in supernatant solution.

In vivo experimental results:

Biochemical results:

Table (1) showed the activity of MAD and antioxidant-related enzymes in serum. Exposure to $Pb(NO_3)_2$ detrimental to the redox status in serum as evidenced by a significant rise ($p< 0.05$) in MAD level and significant depletion ($p< 0.05$) in SOD, GPX and CAT activities in rats with corresponding to control. As compared to the group administered $Pb(NO_3)_2$ only, groups treated with both doses of nano-HAp (150 & 300mg/kg b.w.) restored the activity of SOD, GPX, CAT and MAD particularly, after 48 h of treatment.

Table (1): Effect of intravenous nano-HAp on antioxidant status of rats injected with LD_{50} of $Pb(NO_3)_2$.

Groups / Parameters	Control	$Pb(NO_3)_2$		$Pb(NO_3)_2$ + nano-HAp (150mg/kg)		$Pb(NO_3)_2$ + nano-HAp (300mg/kg)	
		6h	48h	3h	48h	3h	48h
SOD (u/ml)	$3.7^{a}\pm0.34$	$3.1^{b}\pm0.21$	$2.6^{c}\pm0.22$	$3.1^{b}\pm0.11$	$3.3^{ab}\pm0.23$	$3.3^{ab}\pm0.43$	$3.6^{a}\pm0.24$
GPx (mu/ml)	$75.3^{b}\pm6.11$	$54.7^{a}\pm4.44$	$43.0^{c}\pm5.63$	$57.8^{a}\pm3.98$	$58.5^{ad}\pm4.34$	$60.9^{d}\pm6.11$	$65.0^{de}\pm5.32$
CAT (mu/ml)	$7.7^{d}\pm0.21$	$6.2^{b}\pm0.76$	$5.5^{c}\pm0.73$	$6.6^{ba}\pm0.55$	$6.9^{d}\pm0.61$	$6.8^{a}\pm0.28$	$7.1^{d}\pm0.51$
MAD(mmol/l)	$3.6^{a}\pm0.51$	$4.6^{d}\pm0.11$	$5.8^{c}\pm0.41$	$4.2^{b}\pm0.49$	$3.8^{a}\pm0.32$	$4.0^{ab}\pm0.54$	$3.5^{a}\pm0.11$

Values represent means ± S.E.

Values bearing different superscript in the same raw are statistically different.

As shown in table (2), exposure to LD_{50} of $Pb(NO_3)_2$ induced a significant augmentation ($p< 0.05$ & $p< 0.01$) at 6 and 48 h in arginase, GGT, AST and ALT levels, as compared to control animals. Compared to group received lead nitrate only, intravenous nano-HAp gel with both concentrations (150 and 300mg/kg b.w.) reduced but not restored these enzymes levels as shown in table (2).

Table (2): Effect of intravenous nano-HAp on serum liver enzymes of rats injected with LD_{50} of $Pb(NO_3)_2$

Groups / Parameters	Control	$Pb(NO_3)_2$		$Pb(NO_3)_2$ + nano-HAp (150mg/kg)		$Pb(NO_3)_2$ + nano-HAp (300mg/kg)	
		6h	48h	3h	48h	3h	48h
Arg (u/l)	$5.61^{c}\pm0.34$	$7.5^{b}\pm0.41$	$8.5^{a}\pm1.0$	$7.2^{b}\pm0.94$	$6.7^{d}\pm0.66$	$6.8^{d}\pm0.65$	$6.0^{c}\pm0.40$
GGT(u/l)	$53.8^{a}\pm3.8$	$70.4^{b}\pm5.3$	$84.1^{c}\pm5.50$	$66.9^{bd}\pm3.44$	$58.2^{a}\pm5.21$	$60.3^{d}\pm3.60$	$57.3^{a}\pm2.11$
AST(u/l)	$256.7^{b}\pm6.53$	$358^{a}\pm6.80$	$416.9^{c}\pm9.21$	$341.9^{ad}\pm7.0$	$300.1^{bd}\pm4.29$	$308.9^{d}\pm4.75$	$290.2^{bd}\pm3.79$
ALT(u/l)	$118.1^{a}\pm4.81$	$156^{b}\pm3.62$	$176.8^{bc}\pm3.77$	$150.8^{b}\pm3.67$	$139.5^{d}\pm2.53$	$130.3^{ad}\pm2.48$	$131.1^{ad}\pm2.87$

Values represent means ± S.E.

Values bearing different superscript in the same raw are statistically different.

Table (3) clarified that the injection of $Pb(NO_3)_2$ to rats induced significant increase ($p< 0.05$) in serum urea at 6 and 48 h and in serum creatinine after 48 h as compared to control rats. The treatment with both doses of nano-HAp followed lead nitrate ameliorated serum urea and creatinine after 48 h and this effect was more pronounced in the group treated with high dose.

Table (3): Effect of intravenous nano-HAp on serum kidneys function of rats injected with LD_{50} of $Pb(NO_3)_2$.

Groups / parameters	Control	$Pb(NO_3)_2$		$Pb(NO_3)_2$ +nano-HAp (150mg/kg)		$Pb(NO_3)_2$ +nano-HAp (300mg/kg)	
		6h	48h	3h	48h	3h	48h
Urea (mg/dL)	$28^{c}\pm3.71$	$41.5^{b}\pm4.23$	$65.3^{a}\pm2.98$	$45^{b}\pm2.83$	$41.5^{b}\pm3.22$	$40^{b}\pm3.67$	$35^{c}\pm2.94$
Creatinine(mg/dL)	$0.9^{a}\pm0.06$	$1.1^{a.}\pm0.05$	$1.5^{b}\pm0.09$	$1.1^{a}\pm0.16$	$0.8^{c}\pm0.21$	$1.1^{a}\pm0.01$	$1^{a}\pm0.02$

Values represent means ± S.E.
Values bearing different superscript in the same raw are statistically different.

As shown in table (4), administration of $Pb(NO_3)_2$ induced significant increase ($p< 0.01$) in serum corticostrone level in rats after 6 and 48 h as compared to control rats. Intravenous injection of nano-HAp (150 &300mg/kg) followed exposure to LD_{50} of $Pb(NO_3)_2$ inhibit the level of serum corticosterone and such effect was more evident in rats received low dose of nano-HAp.

Table (4): Effect of intravenous nano-HAp on corticoesterone level of rats injected with LD_{50} of $Pb(NO_3)_2$.

Groups	Control	$Pb(NO_3)_2$		$Pb(NO_3)_2$+ nano-HAp (150mg/kg)		$Pb(NO_3)_2$+ nano-HAp (300mg/kg)	
		6h	48h	3h	48h	3h	48h
Corticoesterone	$160^{a}\pm4.32$	$268^{b}\pm5.23$	$218.5^{c}\pm6.82$	$150^{a}\pm3.11$	$155^{a}\pm3.84$	$178^{ad}\pm3.47$	$185^{ad}\pm5.14$

Values represent means ± S.E.
Values bearing different superscript in the same raw are statistically different.

As shown in table (5), rats exposed to LD_{50} of $Pb(NO_3)_2$ showed significant decrease ($p< 0.05$) in serum calcium level after 24 and 48 h., but the level of phosphorus not affected. Both doses of nano-HAp followed $Pb(NO_3)_2$ did not affect either calcium nor phosphorus levels among all treated groups.

Table (5): Effect of intravenous nano-HAp on serum calcium and phosphorus value of rats injected with LD_{50} of $Pb(NO_3)_2$.

Groups / parameters	Control	$Pb(NO_3)_2$			$Pb(NO_3)_2$+nano-HAp (150mg/kg)			$Pb(NO_3)_2$+nano-HAp (300mg/kg)		
		6h	24h	48h	3h	24h	48h	3h	24h	48h
Ca (mg/dL)	$10.9^{a}\pm1.2$	$10.7^{a}\pm0.9$	$9.4^{b}\pm0.4$	$8.3^{c}\pm0.1$	$10.4^{a}\pm1.2$	$9.0^{b}\pm0.1$	$8.7^{c}\pm0.4$	$10.4^{a}\pm0.9$	$9.7^{b}\pm0.3$	$8.9^{cb}\pm0.7$
P (mg/dL)	$7.6^{c}\pm0.6$	$7.5^{c}\pm0.5$	$7.3^{c}\pm0.2$	$7.4^{c}\pm0.1$	$7^{ac}\pm0.1$	$6.9^{ac}\pm0.1$	$7.3^{c}\pm0.05$	$7.1^{ac}\pm0.04$	$7.2^{c}\pm0.2$	$7.4^{c}\pm0.3$

Values represent means ± S.E.
Values bearing different superscript in the same raw are statistically different.

Histopathological results:

Liver tissue:

Severity of the reaction in liver of different groups according to the histopathological alterations treatment.

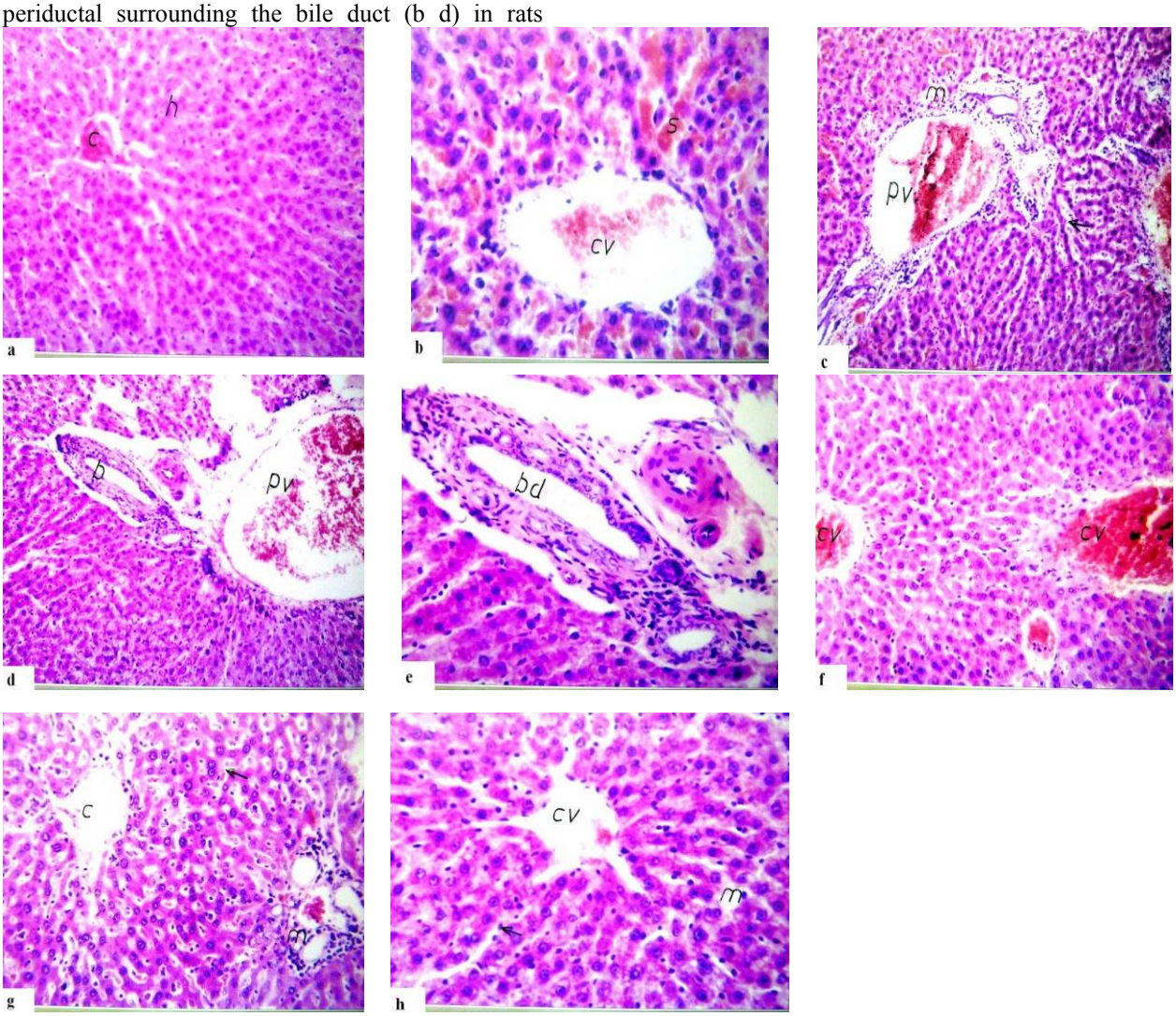
Control	$Pb(NO_3)_2$		$Pb(NO_3)_2$ + nano-HAp (150mg/kg)		$Pb(NO_3)_2$ + nano-HAp (300mg/kg)	
	3h	48 h	3h	48h	3h	48h
-	++	++++	++	+	++	+

Very sever ++++, sever +++, moderate ++, mild +, nil -

Transverse section of control rat liver showed normal histological structure of the central vein (C) and surrounding hepatocytes (H) with centrally located nuclei were recorded (Fig.7a). Rats exposure to lead nitrate (93mg/kg b.w.) induced sever congestion in the portal veins (pv) and sinusoids (arrow) with inflammatory cells infiltration (m) in portal area through 6 h (Fig. 7b). After 48 h, this

congestion associated with other histopathological changes including disruption of the normal structural organization of the hepatic lobules, loss of the characteristic cord-like arrangement of the normal liver cells, vacuolization and infiltration of inflammatory cells (Fig. 7c).

According to histopathological results, treatment with both doses of nano-HAp post $Pb(NO_3)_2$ administration diminished the majority of histopathological changes in the portal area after 3 h. There was a moderate congestion in the central vein (p v) and sinusoids associated with thickening (h) in periductal surrounding the bile duct (b d) in rats received low dose (Fig. 7 d & e) and few inflammatory cells infiltration (m) in the portal area in rats received high dose (Fig.7 f). On the other hand, treatment with nano-HAp post $Pb(NO_3)_2$ administration restored normal arrangement of hepatocytes through 48 h. But, there was a congestion in the central vein (c) in rats received 150mg/kg nano-HAp (Fig.7 g) and proliferation of diffuse kupffer cells (arrow) with infiltration of inflammatory cells in between the hepatocytes in rats received 300mg/kg nano-HAp (Fig.7 h).



(Fig. 7), (H&E)

Fig. (7): Section of rat's liver obtained as follows:
(a) control rats *(x 64)* (b): after 6h of $Pb(NO_3)_2$ administration *(x 80).*
(c): after 48 h of $Pb(NO_3)_2$ administration *(H&E x 40).*
(d &7e): after 3 h of low dose of nano-HAp post $Pb(NO_3)_2$ administration *(x40 &80).*
(f): after 3 h of high dose of nano-HAp post $Pb(NO_3)_2$ administration *(x64)*
(g): after 48 h of low dose of nano-HAp post $Pb(NO_3)_2$ administration *(x64).*
(h): after 48 h of high dose of nano-HAp post $Pb(NO_3)_2$ administration *(x64).*
Kidney tissue:

Severity of the reaction in kidney of different groups according to the histopathological alterations treatment.

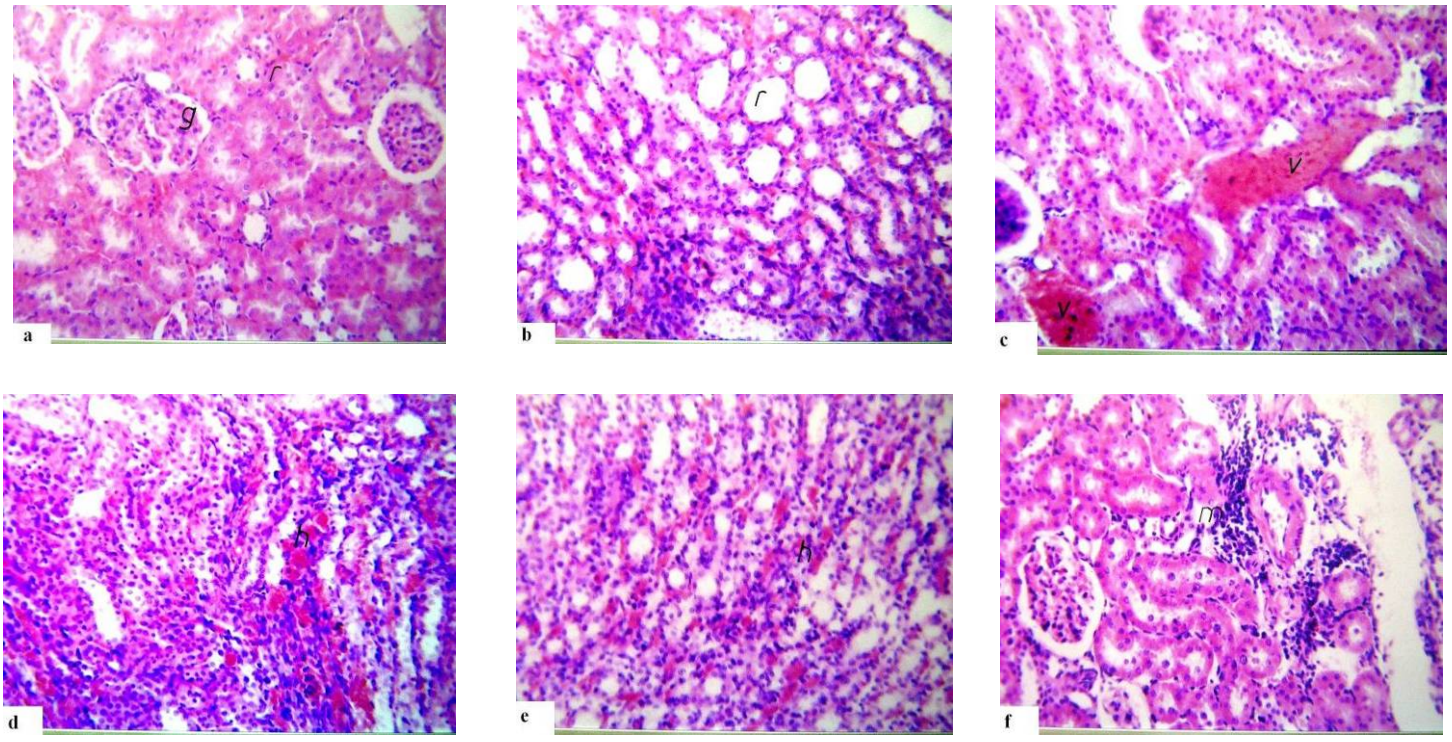
Control	$Pb(NO_3)_2$		$Pb(NO_3)_2$+ nano-HAp (150mg/kg)		$Pb(NO_3)_2$+ nano-HAp (300mg/kg)	
	3h	48 h	3h	48h	3h	48h
-	++	++++	++	++	++	+

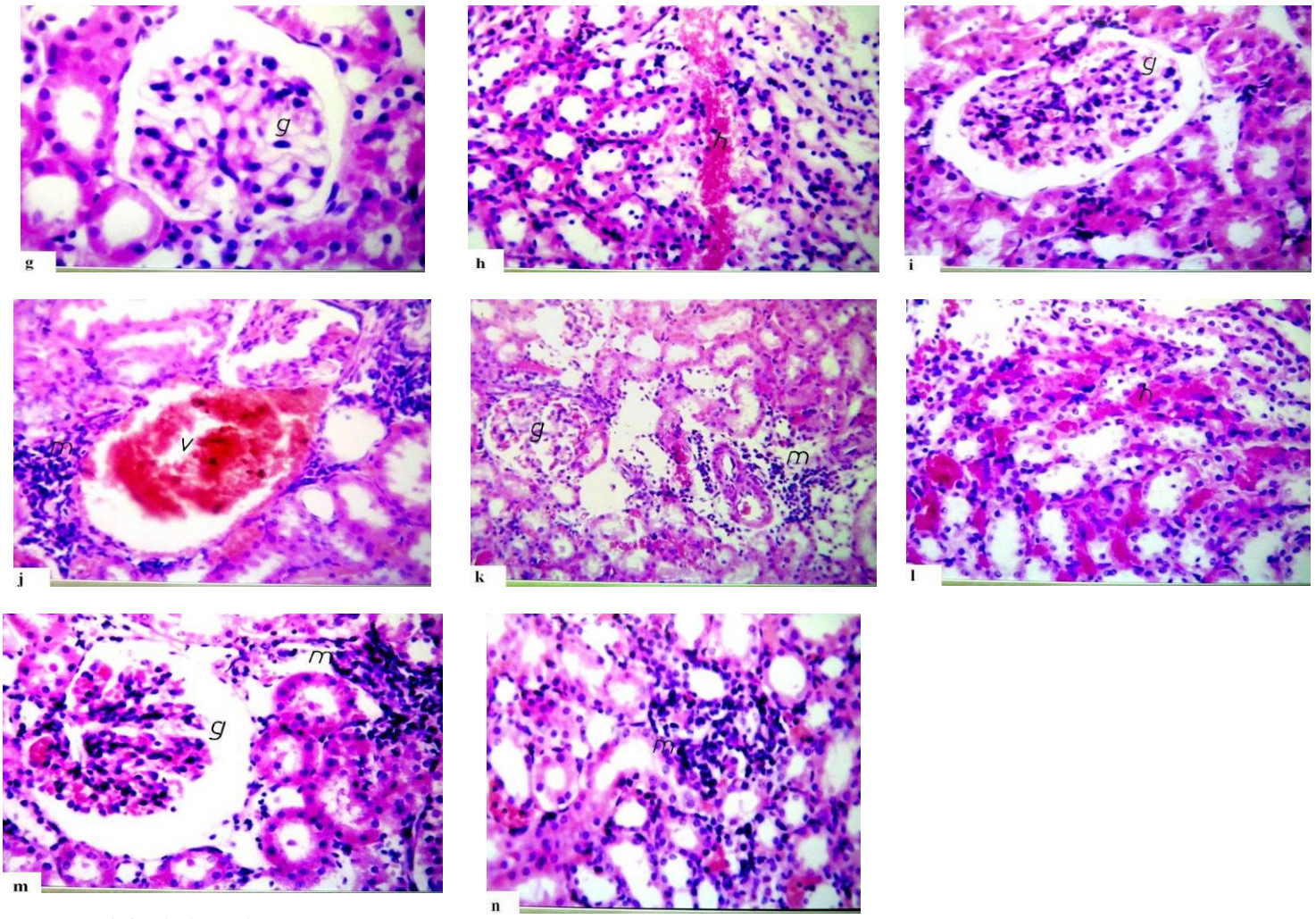
Very sever ++++, sever +++, moderate ++, mild +, nil -

Kidney of control rat showed normal histological structure of the glomeruli (g) and tubules (r) in the cortex (Fig. 8a) as well as the tubules in the medulla (Fig. 8b).

Histopathological investigation of kidney obtained from rats after 6 h of $Pb(NO_3)_2$ administration showed congestion in the cortical blood vessels(v) as shown in (Fig. 8c) associated with focal hemorrhages in the corticomedullary and medulla portions (h) (Fig. 8d&e). After 48 h of $Pb(NO_3)_2$ administration, focal inflammatory cells infiltration surrounding the cortical blood vessels (Fig 8f) associated with swelling and vacuolization of the endothelial cells lining the tuft of the glomeuli (g) were observed (Fig.8g). In addition to focal hemorrhages (h) in the corticomedullary portion (Fig.8h).

According to histopathological results, kidney collected from rats after 3 h of nano-HAp treatment revealed infiltration of focal inflammatory cells in the perivascular (m) area in the cortex associated with congestion in the tufts of the glomeruli (g). But, the focal hemorrhage (h) in the medulla was reduced (Fig 8 I, j & k). On the other hand, after 48 of 150mg/kg nano-HAp treatment, the congestion in the cortical blood vessels as well as focal haemorrhages (h) in the corticomedullary and medullary portions disappeared. But, there was a proliferation of the endothelial cells lining the hyperemic tuft of the glomeruli (g) (Fig8 l). Regarding to the rats received 300mg/kg nano-HAp, there was a proliferation in the endothelial cells lining the hyperemic tufts of the glomeruli associated with focal inflammatory cells infiltration in between the tubules (m) at the cortex (Fig 8 m) as well as in the medulla (Fig 8 n).





(Fig.8), (H&E)

Fig. (8): Section of rat's liver obtained as follows:
(a): kidney cortex of control rats *(x 64).*
(b): Kidney medulla of control rats *(x 64).*
(c & d & e): after 6h of $Pb(NO_3)_2$ administration *(x64)*
(f, g , h): after 48h of $Pb(NO_3)_2$ administration *(x64, x160, x64).*
(i): after 3 h 3 h of low dose of nano-HAp post $Pb(NO_3)_2$ *(x80).*
(j & k): after 3 h of high dose of nano-HAp post $Pb(NO_3)_2$ administration *(x64, x 80).*
(l): after 48 h of low dose of nano-HAp post $Pb(NO_3)_2$ administration *(x80)*
(m & n): after 48 h of high dose of nano-HAp post $Pb(NO_3)_2$ administration *(x80).*

4. Discussion:

Hydroxyapatite is a unique substance because of its high capacity in removal of heavy-metal ions and high biological compatibility (Ozawa and Kanahara 2005). Several investigations concerned the metal ions chelating mechanisms suggested that HAp removed lead ions by ions exchange or by absorption (Admassu and Breese, 1999) or adsorption (Stötzel *et al*. 2009). It was reported that synthetic hydroxyapatite could reduce aqueous pb from 1000 mg/l to less than 1 mg/l (Shashkova *et al*. 1988).

The present results showed that no presence of Pb^{2+} or Ca^{2+} in the solution (Fig. 6) and according to ICP-MS analysis, the increased of nano-HAp concentration had no effect in the decreased of Pb^{2+}. The TEM analyses clarified the trapping of lead nitrate in the polymeric matrix of the formed nano-HAp gel which decreased its crystallinity in FTIR analysis (Fig.3). The same results had been investigated in details for the removal of another divalent ions (Ni^{+2}) and its anion using nano-HAp by Abdelfattah *et al* (2006). Also nano size of the used gel enhanced the capacity of ions removal besides the

presence of PVAL work as steric entrapment for cations leading to complete removal of the elements in the solution. Thus, it could be suggested that nano-HAp gel achieved success in chelating lead ions with its anion and HAp kept its crystals structure with no replacement of calcium ions.

The performance of $Pb(NO_3)_2$ toxicity on oxidant/antioxidant system *in vivo* was manifested in the present study by increase in MDA level and decrease in the endogenous antioxidant enzymes such as SOD, CAT, and GPx. One general mechanism was proposed for lead intoxication through generation of free radicals by either or both of the following events: depletion of glutathione or inhibition of sulfhydryl-dependent enzymes (Silbergeld *et al.* 2000) or interfering with some essential metals needed for antioxidant enzyme activities (Slater, 1985). Subsequently, cellular concentrations of hematoproteins lowered and the redox buffering capacity of cells reduced. Under such conditions, ROS generated by other events may not be neutralized and thereby the likelihood of oxidative damage to DNA may be increased (Caylak *et al*. 2007).

The maximum level of Pb in the liver reached rapidly within the first hour after administration which in turn, increases the susceptibility of cells to oxidative attack by altering the fatty acid composition and membrane permeability resulting in escaping of enzymes from cells into blood (Bechara, 2004). Lead oxidative hepatocytes damage had been responsible for the elevation of serum arginase, GGT, AST and ALT levels observed in the present study. These enzymes considered as biomarkers for liver function and integrity (O'Brien, 2002) and usually elevated in acute hepatotoxicity or mild hepato-cellular injury (Sharma *et al*. 2010). As urea production in mammals occurs essentially in the liver, the plasma concentration of this compound resulting from amino acids catabolism, could also be used as an indicative of hepatic function (Araújo *et al.,* 2005). In addition, kidney cortex and medulla followed the liver among the soft tissues affected by lead exposure (Nolan and Shaikh 1992). However, kidney disease (nephropathy) is a characteristic manifestation of lead toxicity. Thus, elevation of creatinine level observed in $Pb(NO_3)_2$ treated group may be due to loss of 50% of kidney function and referable to functional evidence of lead induced nephrotoxicity (Qu *et al*. 2002). Accordingly, it could be postulated that lead-induced oxidative stress and lipid peroxidation with concomitant inhibition of several antioxidant enzymes in blood (Bolin *et al*. 2006 and Arif *et al.* 2008) has been to be one of the possible mechanisms of its toxic effects (Pande and Flora, 2002) and major contributors to lead-exposure related diseases (Patrick, 2006).

Regarding to corticosterone, the elevation in its level after exposure to $Pb(NO_3)_2$ was a detrimental effect related also to oxidative stress. This is because exposure to lead is well known by association with inflammation mediated by oxidative stress (Songdej *et al.* 2010) which induced hyperactivity of hypothalamo-pituitary-adrenal axis yielding elevated corticoesterone level (Parthasarathy *et al.* 2006). On the other wise, lead has adverse effect on immune system and decreased antibody for matrix inflammatory response (Sin and Woo, 1992). These findings support the concept that susceptibility of rats to lead was regulated by HPA axis-immune system feedback loop responsiveness to inflammatory stress.

It is pertinent to mention that the peak concentration of intravenous lead in blood is reached about one hour after injection (35 to 40% of the administered dose) in the rats. The level in blood declined fairly rapidly thereafter and only about 5% of the dose remained in blood after two days (Poirier *et al*. 2006). Lead reacted rapidly with calcium and phosphors in plasma and caused a withdrawal of these ions indirectly. But calcium and phosphorus are immediately withdrawn from extravascular sources returning plasma concentrations to their initial value (Talmage *et al.* 1978). At the same time, the most concentration of intravenous nano-HAp detected in liver and spleen at 1 h after administration and decreased significantly after 72h (Tang *et al.* 2009 and Xie, 2010). Thereby, calcium and phosphorus levels monitored in the present study at times intervals of 6, 24 and 48 h post $Pb(NO_3)_2$ administration to clearly detect the performance interaction of i.v. nano-HAp with $Pb(NO_3)_2$ related to concentration of both metals in the blood. The results revealed gradual decrease in calcium level after 24 h forward to 48 h post $Pb(NO_3)_2$ administration while phosphorus level not affected. Such decrease in calcium level was probably because lead often shared calcium transport mechanisms, through the physicochemical interactions at the injection site or through biochemical competition at critical macromolecular binding sites (Poirier *et al.*, 2006).

The most important renderings of nano-HAp gel bioavailability observed in the present study was improvement of the antioxidant enzymes and lipid peroxidation status because the oxidative stress is the manager for internal and/or external factors induced adverse effect. Such antioxidant effect of nano-HAp may be attributed to the scavenging of superoxide, which is the main component of oxidative stress (Scherbart *et al*., 2009) or to inhibition of oxidative and nitrooxidative species formation (Fouda *et al*.,

2009). However, elevated activity of catalase is more beneficial than increase in SOD activity alone because without a simultaneous increase in catalase activity, increased SOD activity may lead to intracellular accumulation of H_2O_2 with detrimental effects (Das *et al*., 1995).

On the other hand, the results of liver and kidney functions contained a lot of important information. Since, all the nano-HAp treated animals have normal kidney function due to normal creatinine levels. Also, the survival of all the animals through the experiment indicated that the liver was not seriously damaged or not fatal (Jayabalan *et al.,* 2010). In this aspect, Xie (2010) investigated the quantitative tissue distribution of intravenous nano-HAp in rats using ^{125}I radiolabeling and reported that nano-HAp accumulated in the soft tissues mainly liver and spleen. Apparently, nano-HAp having antioxidant activity effective in treating $Pb(NO_3)_2$ induced hepatotoxicity by scavenging the free radicals, thereby preventing the liver and kidney damage induced by both $Pb(NO_3)_2$ as well as subsequent depletion of glutathione.

There is inconsistency between the general view of literature and the present results because the degradability of intravenous nano-HAp not as stated before when injected after intraperitoneal lead nitrate. This proposal based on the mentioned results referable to similarity of serum calcium level among the rats treated with either nano-HAp and $Pb(NO_3)_2$ or lead nitrate only treated rats. Additionally, internalization of i.v. nano-HAp inside the body was confirmed by the recovery of liver and kidney function and structure as well as corticosterone level. Thus, it could be in the light of the *in vitro* investigation data suggested that nano-HAp uptake lead by capture it in the blood, liver and kidney.

Histopathological examination revealed that induction of hepatic and renal cellular proliferation occurred with single dose of lead nitrate administration. A growing body of evidence suggests that oxidative stress is the key player in the pathogenesis of lead-induced toxicity (Adamis *et al*., 1999). It was shown that, lead nitrate induced a synchronized wave of hepatocyte proliferation in rat liver without accompanying histopathological necrosis (Abdel-Aal *et al.,* 1989). Moreover, Proliferation on non parenchymal cells reflect a direct mitogenic effect of lead nitrate and that hepatocyte proliferation follows the nonparenchymal cell reaction to lead nitrate (Kubo et al., 1996). In this view Pezzatini *et al.* (2006) showed that lead-induced liver hyperplasia followed by apoptosis mediated by oxidative stress in kupffer cells. Rijhsinghani *et al.,* (1993) have reported that 8 hours after an intravenous injection of lead nitrate, DNA synthesis was detected in a few scattered hepatocytes and in nonparenchymal cells, including bile duct epithelial cells, fibroblast, macrophage, an nondescript periductular cells.

The histopathological results as biochemical results confirmed the biological efficacy of nano-HAp since, liver and kidney restored most of normal structure after the treatment with nano-HAp. It was clear that the nano-HAp particle size could influence the pathway of internalization, the increase of the inhibition effect on the proliferation activity could be reduced (Bauer *et al.,* 2008).

General observations favoring enzyme release during reversible cell damage include the apparent lack of histologic evidence of necrosis in spite of increased serum enzyme activity. Conceivably however, this may also reflect a lack of sensitivity of histologic techniques to detect cell necrosis when it is patchy, early on, or involves only a small number of cells. Nevertheless, additional findings made over the previous decades have led to a growing acknowledgement that the escape of enzymes from cells likely includes mechanisms other than cell death. While increased membrane permeability is a well-known outcome of irreversible cell damage, it is unlikely that cells sustaining the formation of perforations or tears of adequate size to allow the leakage of macromolecules such as enzymes could maintain adequate viability to recover. The mechanism proposed to explain the appearance of cytosolic enzymes in blood with reversible damage is by the formation of membrane blebs that detach and allow the cell membrane to reseal without cell death (Mair, 1999).

5. Conclusion:

Nano-HAp gel was synthesized and tested as of prospective chelating agent of one of very toxic heavy metal. The nano-composite gel successfully removed lead ions and its anion from its solution. The lead ions were completely chelated and intervened with the polymeric gel reducing the degree of HAp crystallinity as being completely adsorbed on the HAp structure with its anion. The newly-formed nano-HAp gel have shown good biocompatibility and little toxicity when injected intravenously. It showed significant and fast therapeutic effect of lead nitrate toxicity within 48h. Apparently, the biological risks, cytotoxicity of the HAp nanoparticles are size-and dose-dependent. The future application of nano-HAp gel in the human biomedical was expected. Further study is needed to be carried out in order to proof its safety and efficacy in the human body.

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Silver nitrate staining improves visual analysis of daily otolith increments

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Abstract: Sagittal otoliths in juvenile to sub-adult (62mm-150mm standard length) gray snapper (*Lutjanus griseus*) were analyzed using a modified staining method. Daily growth increments from transversely sectioned otoliths were stained using silver nitrate and fixed using sodium thiosulfate. Stained otoliths showed a noticeable improvement in the resolution of daily increments compared to those not stained. This procedure lends to the enhanced visualization of daily rings and has the potential to be a timely, yet efficient, technique for age and growth analysis of calcium carbonate structures.

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Keywords: silver nitrate, staining, otolith, daily increment, von Kossa

1. Introduction

An essential component of fisheries science is the knowledge and understanding of age and growth of fishes. There are numerous ways to determine age and growth of fishes; however, to discern the age of wild fish, it is imperative to analyze calcified structures. Otoliths are one of these structures and they hold a wealth of information on daily age, size, growth and ontogeny of fishes. They are the most reliable indicators of fish age simply because otoliths are the only structures that consistently record daily events in the early life stages (Campana and Jones, 1992). Otolith microstructure analysis is the preferred means to determine age and growth of fishes because experimental evidence reveals that otolith material is neither re-absorbed nor reworked after deposition (Thorrold and Hare, 2002). Although otolith microstructure increments differ in shape and pattern depending on the fish family and species, one thing remains constant: otoliths serve as a permanent record for changes in environmental conditions and physiological changes experienced by the fish. The literature discussed here refers to reef fish, a category into which gray snapper fit.

Otoliths are calcareous accretions found within the semicircular canals of teleost fishes. They are organized in alternating layers dominated by hydrophobic high-molecular-weight proteins and inorganic calcium carbonates (Thorrold and Hare, 2002). Otoliths are used for balance and hearing of fishes and are useful tools for age determination through analysis of annual and daily increments (Campana, 2001). Daily increments occur as a result of the endogenous circadian rhythm occurring in fish (Campana and Jones 1992). The formation of daily increments takes place under most conditions, including times of food deprivation. In the absence of any somatic growth, deposition is maintained because the endolymph chemistry is buffered from the blood plasma (Thorrold and Hare, 2002). The production of visible daily increments is contingent upon a daily cycle of differing rates of accretion of a protein matrix and crystalline inclusions (Victor, 1991).

Determination of the age of fish has represented a challenge for otolith readers, and various methods have been used to improve visualization of annuli. Successful otolith age determination techniques require the appropriate otolith preparation method. The method of choice is largely dependent on the size of the otolith and the type of analysis to be performed. The most popular preparation method involves simply mounting and clearing small otoliths that are less than 50 µm in diameter (Secor et al, 1992). This method enables adequate resolution of daily increments on otolith microstructure. For larger otoliths, sectioning and polishing is required in order to remove material and expose the core and all increments.

The von Kossa staining method has been utilized in fish age studies for decades, including the use of whole vertebrae, neural arches (McFarlane et al 2002, Rossouw 1984), and in the age determination of not only teleosts but also elasmobranchs (Stevens 1975, Cailleiet et al 1983, Green et al., 2002). In this study, we describe a new method to improve the visualization of otolith increments, thereby making it easier to count during age estimation. This new method is silver nitrate ($AgNO_3$) staining of otoliths, derived from Von Kossa's staining method for calcium. The positive silver ions from $AgNO_3$ bind with the negative carbonate ions (CO_3^{-2}) in Calcium Carbonate ($CaCO_3$).

This bond darkens the increments, or "stains" them, and allows the increments to be viewed much more easily. Previous studies experimented with dyeing, burning and staining techniques (Richter and McDermott, 1990; Bouain and Siau, 1988).

2. Materials and Methods

Juvenile gray snapper (131mm SL) otoliths were embedded in resin and cut with an Isomet low speed saw, resulting in transverse sections approximately 1 mm thick. A 2.943mM of $AgNO_3$ and a 3.162mM of Sodium Thiosulfate ($Na_2S_2O_3$) solution were prepared and a series of Petri dish baths were assembled in the following order: distilled water, $AgNO_3$ solution, distilled water, $Na_2S_2O_3$ solution, and distilled water. Each transverse section was dipped in the distilled water bath, and then placed in the $AgNO_3$ solution for ten minutes. The otolith section was then introduced to the next distilled water bath. Subsequently, otolith sections were individually illuminated with ultraviolet light (15 W) for fifteen minutes. Otoliths were then dipped in $Na_2S_2O_3$ solution for two minutes to allow the silver stain to become affixed to the "stained" otoliths and to remove excess silver (Stevens, 1975). Finally, stained otoliths were dipped in the last distilled water bath before air drying on a flat surface. Stained otolith sections were glued to a microscope slide using thermoplastic glue and were polished using 800 and 1200 grit sandpaper until the otolith core was observed clearly with a compound microscope at 40x magnification. Daily increments were examined while focusing at magnifications of 40-200x.

In order to quantify the impact of staining otoliths, a sagittal otolith was processed, photographed and analyzed, and was subsequently stained and photographed again. Digital micrographs with transmitted light were taken using a compound microscope and Image Pro 4.5 with digital camera Evolution MP at 200x. Lighting was maintained at the same setting and no additional adjustments or enhancements were made to either image. Using Image Pro software, the images were scaled and line profiles were drawn from the edge towards the core while the line was perpendicular to the daily increments. The line profile feature measures the intensity of pixel values along the feature (Image Pro 4.5 user guide, 2002) and can be used to identify peaks or maximum values (darker) and troughs or minimum values (lighter) in otolith age determination. Adjacent minimum and maximum values in intensity across the otolith were used to identify peak and trough locations. The value for each peak and trough was extracted, and then subtracted from the transect mean. To investigate whether the silver nitrate treatment had enhanced the contrast across the treated otolith, and thus magnified the differences between adjacent peaks and troughs, the difference between each peak and the previous trough (or each trough and the previous peak) was calculated. The minimum, maximum and standard deviation of these values were then used to determine if silver nitrate staining enhanced extreme peak and trough values, and if differences between adjacent peaks and troughs increased, as measured by the standard deviation.

3. Results

Silver nitrate treatment appeared to improve the resolution of daily otolith increments (Figure 1). Overall, the line profiles in the stained otolith had higher values for peaks and lower values for the troughs when compared to the unstained otolith. The maximum difference in intensity between adjacent peaks and troughs was also greater in the stained otolith (Figure 2). Standard deviations of differences between adjacent peaks and troughs were higher for the stained otolith, suggesting that silver nitrate treatment had enhanced the contrast between daily growth increments.

4. Discussions

Currently, age determination using otoliths is the most common method for fishes, although the difficulty of counting daily increments varies greatly across different taxa. Although daily increments occur, the length of the increment formation can vary due to several environmental and physiological parameters, thus make daily increment interpretation difficult (Campana and Neilson 1985, Wooton, 1990). The use of silver nitrate to stain juvenile gray snapper otoliths successfully enhanced the visibility of daily increments and improved precision and accuracy when reading otolith increments. Stained daily increments darkened the translucent zones that appear raised under transmitted light while enhancing the contrast of the opaque zones that appear depressed. This allowed for the age of the fish to be more easily determined.

Silver nitrate staining may be used for otolith age determination of different age classes from larval (Green et al., 2002) to adult, and for fishes that inhabit tropical and subtropical environments where daily or annual increments may be difficult to distinguish. This method is inexpensive and has the potential to be implemented for stock assessment studies that monitor the age and growth of large quantities of commercially valuable species.

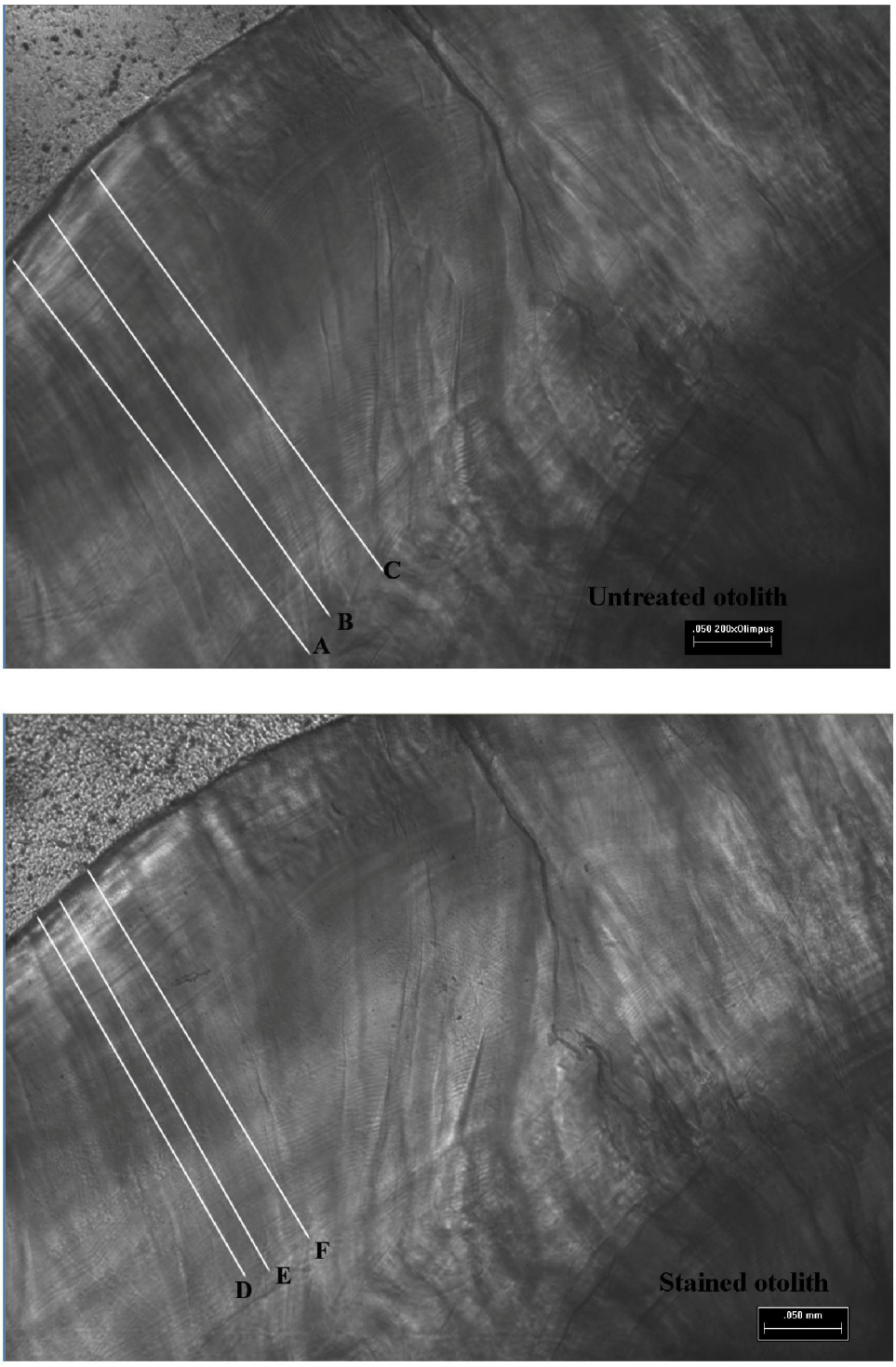
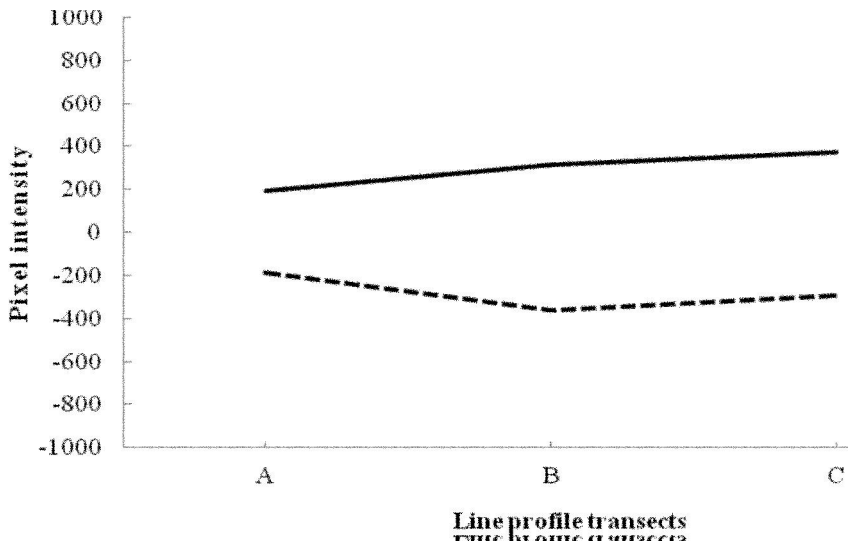


Figure 1: Digital micrographs of one transverse section of a Gray Snapper otolith with lines showing the line profiles used for comparing the intensities of daily increments of untreated (top) and stained with silver nitrate (bottom).

a)



b)

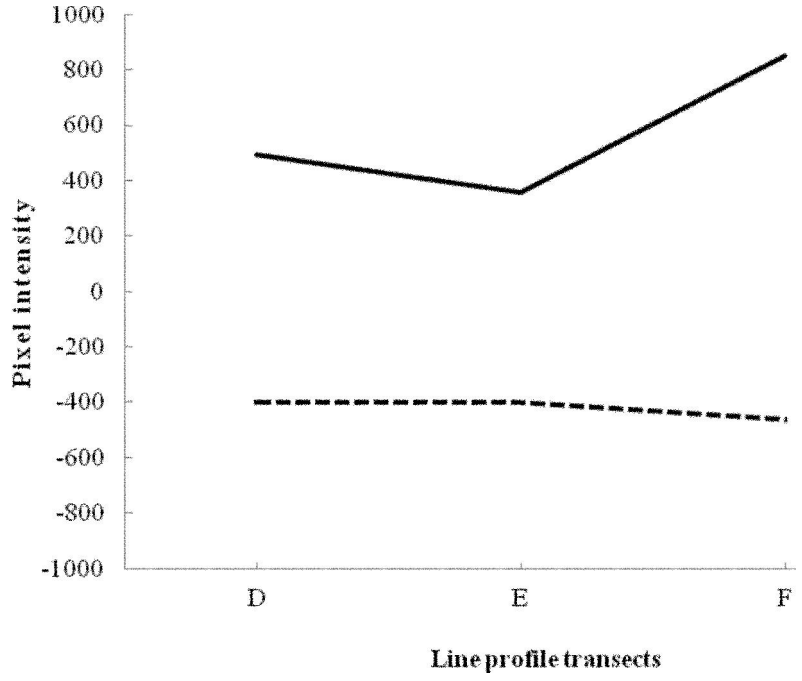


Figure 2. a) Line graph plotting the maximum values (solid line) and minimum values (dashed line) of pixel intensity of untreated line profile transects A, B & C. b) Line graph plotting the maximum values (solid line) and minimum values (dashed lien) of pixel intensity of silver nitrate stained transects D, E &F.

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Arsenic Toxicity in the Irrigation Water-Soil-Plant System: A Significant Environmental Problem

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Abstract: Environmental pollution is a major global concern. When sources of water pollution are enumerated, agriculture is, with increasing frequency, listed as a major contributor. One of the major factors determining uptake and toxicity to plants is the form of arsenic (As). Naturally occurring arsenic in groundwater of sedimentary aquifer has emerged as a global problem, and issue of major environmental concern. It is released and contaminated in agricultural soil by natural weathering, industrial production and mining. However, the same water resources are used extensively for irrigation purposes throughout the region. The two most important forms, As (V) and As (III), are taken up by completely different mechanisms. Uptake, accumulation and toxicity vary within and between plant species. In general, more As in the soil leads to higher concentrations in plants, but this depends on many factors. It is recommended to initiate an integrated program to quantify the scale of the problem in combination with the development of a water-soil-plant quality monitoring system for land degradation in agro-ecosystems. This should not only include As, but a range of physical, chemical (nutrients and contaminants) and biological parameters. Further, management options to prevent and mitigate As contamination need to be explored.
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1. Introduction

Agriculture, as the single largest user of freshwater on a global basis and as a major cause of degradation of surface and groundwater resources through erosion and chemical runoff, has cause to be concerned about the global implications of water quality. If water is polluted, it may be dangerous for plants, animals as well as for human being. The associated agrofood-processing industry is also a significant source of organic pollution in most countries. Development increases, so does the need for water. The water quality in a region largely depends on the nature and extent of the industrial, agricultural and other anthropogenic activities in the catchments (Banejad and Olyaie, 2011). Aquaculture is now recognized as a major problem in freshwater, estuarine and coastal environments, leading to eutrophication and ecosystem damage (Ongley, 1996).

Widespread use of arsenicals as pesticides has significantly contributed to the elevation of arsenic concentrations in soils (Adriano, 2001). Arsenic contamination in groundwater is a severe global environmental problem (Yavuz et al., 2010). Arsenic is a heavy metal with a name derived from the Greek word *arsenikon*, meaning potent. Arsenic is ubiquitous, found in air, water, fuels, and marine life. The daily human intake of arsenic contained in food ranges from 0.5-1 mg, with the greatest concentrations coming from fish and crustaceans. Arsenic has been used for a variety of purposes. For Arsenic and its compound are well known for its toxicity and carcinogenicity (Marcus, 2010). Individual exposes to arsenic from various sources like food, air, water, occupational settings and medicines. Contamination of arsenic in ground water is the global problem and millions of people are at a risk of arsenicosis. Contaminated ground water is the main source of exposure to inorganic arsenic to the human population. Inorganic and organic arsenic occur naturally in the environment, with inorganic forms being most abundant. Inorganic arsenic is associated with other metals in igneous and sedimentary rocks, and it also occurs in combination with many other elements, especially oxygen, chlorine, and sulfur. Organic arsenic contains carbon and hydrogen. Both inorganic and organic forms exist naturally in soils, plants, animals, and humans. Most pure, inorganic arsenic compounds are white or colorless powders with no specific smell or taste. Because it is an element, arsenic does not degrade nor can it be destroyed.

Arsenic is a crystalline metalloid that exists in several forms and oxidation states. Its

toxicity and mobility in the environment depend on both its chemical form and species (Pongratz, 1998). Total metal concentration alone is insufficient to assess its environmental impact in contaminated soils. Risk assessment of contaminants requires information on contaminant pools of differential lability and bioavailability in a soil (Wenzel et al., 2006). Soil available arsenic content is a better indicator of its phytotoxicity than total arsenic concentration (Fayiga et al., 2007). However, available arsenic concentration in soils depends on the type and strength of the extracting agent used (Jain and Ali, 2000).

Arsenic (As) is widely distributed in the environment, originating either from As in the soil parent material or from discharge of As onto land as a result of human activities. Consequently, people and livestock are being exposed to As via contamination of drinking water and consumption of food grown in As-contaminated soil or irrigated with As-contaminated water. Understanding how As is taken up by plants and subsequently transformed in plant tissue is therefore essential for estimating the risks posed to human and wildlife populations by As contaminated soils (Meharg and Whitaker, 2002).

Arsenic (As) is a widespread natural element, which is not a bioorganic element to plants (Stoeva et al., 2003). In terrestrial plants, both organic and inorganic As species have been found (Koch et al., 2000; Francesconi et al., 2002), with the inorganic species (Arsenate [As (V)] and arsenite [As (III)]) being the most dominant. Arsenate is the predominant As species in aerobic soils, whereas arsenite dominates under anaerobic conditions (Smith et al., 1998). Arsenic availability to plants is greatly influenced by its forms in soil. Agricultural application of arsenicals has introduced many different kinds of arsenic compounds to the soil environment. These arsenicals may influence arsenic mobility and plant uptake though they are subjected to oxidation–reduction transformation in soils.

Arsenic is a nonessential element for plants, and inorganic As species are generally highly phytotoxic. Biomass production and yields of a variety of crops are reduced significantly at elevated arsenic concentrations, with application of only 50 $mg.kg^{-1}$ to soil significantly decreasing the yields of barley and ryegrass. Arsenic concentrations are generally low in plants (Matschullat, 2000). The limited accumulation of As by roots and its limited translocation to the shoots, is usually used by most plants such as carrot, tomato and grass. These plants contain relatively low arsenic and accumulate arsenic primarily in their root systems (Matschullat, 2000). In all plant species tested so far, it has been shown that arsenate is taken up via the phosphate transport systems.

1.1. Toxicity of arsenicals

Arsenic is well known for its acute toxicity. For example, an ingested dose of 70-180 mg of arsenic trioxide (As_2O_3) is lethal to humans. Somewhat lower doses produce sub-acute effects in the respiratory, gastrointestinal, cardiovascular, and nervous systems (Jain and Ali, 2000). Chronic exposure to arsenic in drinking water has been linked to serious dermatological conditions, including black foot disease. Epidemiological studies have linked arsenic in drinking water with cancer of the skin, bladder, lung, liver, and kidney (Hindmarsh, 2000) and other ailments. Both As(III) and As(V) are strongly adsorbed in the human body. As(III) tends to accumulate in the tissues, whereas As(V) and organic arsenic are rapidly and almost completely eliminated via the kidneys. The MCL for arsenic in drinking water for many years was 50µg/L, but recent research has suggested that the cancer risk at 50µg/L is unacceptably high. A review of the available arsenic- and health-related data prompted the USEPA to lower the MCL to 10µg/L, the same as the World Health Organization's standard.

1.2. Arsenic and Human Health Effects

Depending on the amount ingested, arsenic can be beneficial (animal studies suggest that low levels of arsenic in the diet are essential) or adverse (high levels can be toxic). The acute lethal dose to humans can be about 2 to 20 mg/kg body weight per day (mg/kg-day). Ingesting high doses of arsenic irritates the stomach and intestines, with symptoms including nausea, vomiting, diarrhea and liver swelling. However, wide recognition of its toxicity makes arsenic poisoning today very rare. Ingesting small amounts over time produces chronic effects such as skin darkening and formation of corns, damage to peripheral nerves, cardiovascular system effects, hair and appetite loss, and mental disorders. Effects from inhaling arsenic dust include respiratory irritation, rhinitis, pharyngitis, laryngitis, and sometimes nasal perforation. Skin contact with inorganic arsenic dusts can cause dermatitis, allergic hypersensitivity, and conjunctivitis. Occupational exposure studies show a correlation between chronic arsenic exposure and lung cancer. Arsenic can also cause reproductive/developmental effects, including spontaneous abortions and reduced birth weights. Epidemiological studies indicate an association between arsenic concentrations in drinking water and increased incidences of skin, liver, kidney, lung, and bladder cancers. Studies also show an association between inhaling arsenic and lung cancer. From these

sets of data, the U.S. Environmental Protection Agency (EPA) has classified inorganic arsenic as a known human carcinogen. Limited information is available on the joint toxicity of arsenic with other chemicals. For neurological effects, the predicted direction of joint toxicity of arsenic and lead is greater than additive, whereas the joint toxicity of these metals is predicted to be less than additive for the kidney and hematopoietic (blood-forming) system. The joint toxicity of arsenic and cadmium on the kidney, hematopoietic system, and male reproductive system is predicted to be less than additive. Additional information on joint toxicity is provided in the companion chemical mixtures fact sheet (Figure 1).

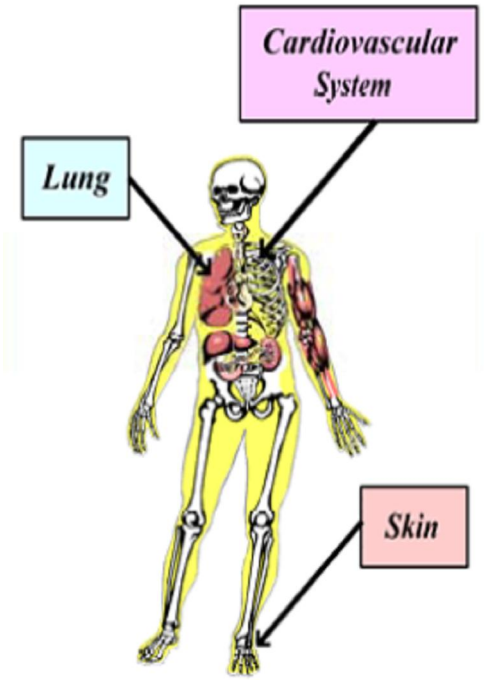

Fig 1. Primary organs affected when arsenic is inhaled or ingested

1.3. Arsenic contaminated irrigation water: the risks

To date, only limited attention has been paid to the risks of using contaminated groundwater for irrigation. Irrigation water with high levels of As may result in land degradation in terms of crop production (loss of yield) and food safety (food chain contamination) (Brammer, 2005; Duxbury et al., 2003). Long-term use of As-contaminated irrigation water could result in As accumulation in the soil. If absorbed by the crops, this may add substantially to the dietary As intake, thus posing additional human health risks. Over time, As accumulation in the soil could reach soil concentrations toxic to crops, thus reducing yields (Figure 2).

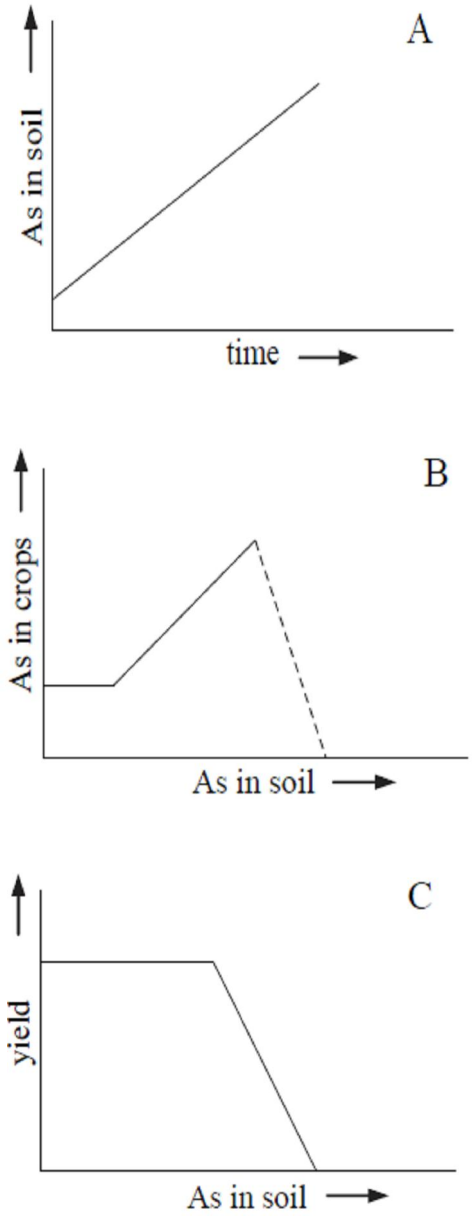

Fig 2. The possible risks of using As-contaminated irrigation water over time.

Note: A: input of As via irrigation water can lead to accumulation of As in the soil over time. B: depending on bioavailability, uptake and transport within the plants, higher soil concentrations may be reflected in higher concentrations in crops. The dotted line indicates that at a certain level the plant growth becomes severely inhibited and As

concentrations in the plants are then no longer relevant. C: with an increase in soil concentration, yields are expected to stay more or less constant until a threshold level is reached, after which yield will decline.

Reliable and representative data are therefore needed to assess and manage the risks of As-contaminated irrigation water.

2. Arsenic in agriculture: current knowledge

2.1.1. Soil Chemistry

Low levels of As are naturally present in the soil (Matschullat, 2000). The background levels are around 5 mg/kg worldwide with substantial variation depending on the origin of the soil (Mandal and Suzuki, 2002). The behavior of As is distinctly different under flooded (anaerobic) and non-flooded (aerobic) soil conditions, with flooded conditions being likely the most hazardous in terms of uptake by plants and toxicity, as will be explained in this chapter. Taking into consideration that rice is the staple crop in Asia, that its cultivation largely takes place under flooded conditions, and that its high demand for irrigation water, often from groundwater resources, understanding the behavior of As under flooded soil conditions is of particular importance.

2.1.2. Arsenics speciation in the soil

As exists in the environment in various organic and inorganic forms (species). The most important inorganic species are arsenate (AsV) and arsenite (AsIII). Monomethylarsenic acid (MMA) and dimethylarsenic acid (DMA) are the most common organic species in the soil, but their natural presence is low compared to inorganic As (Abedin et al., 2002; Fitz and Wenzel, 2002). Speciation of inorganic As in the soil is largely controlled by reduction and oxidation processes (redox). Under aerobic (oxidizing) conditions AsV predominates, whereas AsIII predominates under anaerobic (reducing) conditions (Fitz and Wenzel, 2002; Takahashi et al., 2004). For example, in an experimental paddy field 30 percent of the As was present as AsIII under non-flooded conditions and up to 70 percent was present as AsIII under flooded conditions (Takahashi et al., 2004). Under more reducing conditions, AsIII became by far the predominant species and the solubility of As increased sharply. Microbial activity can influence As speciation via various mechanisms such as redox reactions with Fe and As and via (de) methylation of As species (Fitz and Wenzel, 2002; Mahimairaja et al., 2005).

2. 2. Plants

2.2.1. Uptake

AsIII and AsV are taken up by different mechanisms. AsV is taken up via the high affinity phosphate uptake system (Meharg, 2004). PO_4 additions have therefore been suggested to reduce uptake because of competition between PO_4 and AsV for uptake. For rice grown in pots with soil and irrigated with AsV contaminated water, no effect of PO_4 on As accumulation in rice plants was observed (Abedin et al., 2002). Abedin et al. (2002) suggested that the plants were effectively exposed to AsIII and not to AsV because of the reducing soil conditions. An alternative explanation is that PO_4 competes with AsV both for both sorption at Fe-plaque and for uptake, minimizing the overall effect of PO_4 (Chen et al., 2005). As summarized in various papers, the addition of PO_4 to As-contaminated soils to minimize As uptake is controversial under non-flooded condition (Abedin et al., 2002; Fitz and Wenzel, 2002). AsIII is actively taken up by so-called water channels (aquaporins) in the roots (Meharg and Jardine, 2003). Laboratory experiments have shown that Boro (dry season) rice cultivars take up less AsIII and AsV than Aman (rainy season) rice cultivars. This may be related to physiological or morphological differences between the root systems (Abedin et al., 2002). However, this does not imply that Boro rice will accumulate less As than Aman rice under field conditions, because Boro rice is irrigated with As-rich groundwater whereas Aman rice is rain fed. The uptake mechanism of organic As is largely unclear (Meharg, 2004). It seems that monomethylarsenic acid (MMA) and dimethylarsenic acid (DMA) are taken up by rice plants but that the rate of uptake is much lower compared to inorganic As (Abedin et al., 2002). To date, it has not been possible to predict As uptake by plants from the soil. Most papers only include total As concentrations in the soil and the As concentration in the irrigation water. It has been suggested that total As can be regarded as potentially bioavailable in paddy fields, because most of it is bound to FeOOH. Good correlations between total As in soil and plants are however not always found (Jahiruddin et al., 2005; Miah et al., 2005).

2.2.2. Translocation and accumulation

With the exception of hyper accumulators such as certain ferns, the translocation of inorganic As from the roots to the above ground parts is limited. Organic As is more readily translocated but the uptake is much lower compared to inorganic As (Carbonell *et al.*, 1998). In pot experiments with rice plants exposed to As added via AsV in irrigation water, plant parts were ranked according to the As concentrations as follows: root > straw > husk > grain. Concentrations in all plant parts increased with

the exposure concentration (Abedin *et al.*, 2002). This is a common observation for other plants as well (Bleeker *et al.*, 2003; Carbonell *et al.*, 1998; Sneller *et al.*, 1999b).

3. Soil culture irrigated with As-contaminated water

Abedin et al. (2002) exposed rice cultivar to AsV and studied growth and As uptake. The first observed adverse effect was a reduced root biomass at 0.2 mg/l. Other effects including reduction of plant height, spiklet weight, number of spiklets and grain yield started at 2 mg/l. In an almost similar experimental setup, a reduced root biomss, grain number and grain weight (g/pot; 26 percent reduction) was found at ≥1 mg/l (Abedin et al., 2002). Comparing the two studies suggests that the lowest As concentrations associated with toxic effects deviated substantially despite the similar setup. The main reason is probably the difference in the lowest As concentrations used in the irrigation water, namely 0.2 mg/l in Abedin et al. (2002) and 1.0 mg/l in Abedin et al. (2002). In both studies, first effects occurred already at those levels. This indicates that the range of exposure concentrations did not include a concentration so low that it did not cause any effect. It seems that for this particular experimental setup, the lowest concentration causing adverse effects is equal to or below 0.2 mg/l. Smith et al. (1998), cited in Abedin et al. (2002), reported that rice, bean, oats can suffer from phytotoxicity at a soil concentration of 20 mg/kg, whereas for maize and radish this is 100 mg/kg. According to Sheppard (1992), also cited in Abedin et al. (2002), soil type is the most important variable for toxicity of inorganic As to plants, with soil texture one of the most important factors. Inorganic As was five times more toxic in a sandy soil (40 mg/kg) than in a clayey soil (200 mg/kg). Yan-Chu (1994), also cited in Abedin et al. (2002) found a rice yield reduction of ten percent at 13 and 23 mg/kg soil. In sandy soil with 47–52 mg/kg, rice growth was reduced by up to 50 percent and completely inhibited at 109–157 mg/kg soil. Islam et al. (2004) carried out a similar experiment with the same soil and rice cultivars as Jahiruddin et al. (2004) with the difference being that AsV was added via irrigation water during Boro rice cultivation in the Islam et al. experiment. During the Aman cultivation As-free irrigation water was used, resembling the field situation. With an increase in As concentration in the irrigation water, first an increase in grain yield was observed, both for Boro rice and Aman rice. After that, yields declined (Figure 3). As concentrations in grains steadily increased with As levels in irrigation water (Figure 3). Within the tested range of As concentrations in irrigation water, the observed toxic effects and As accumulation in grains reported by Islam et al. (2004) were far less compared to the observations within the range of soil concentrations used by Jahiruddin et al. (2004). At first, the patterns seem to differ, but a closer look reveals that it is most likely that the range of concentrations used by Islam et al. (2004b) was narrower than that used by Jahiruddin et al. (2004). Comparing the two sets of results for 0–10 mg/kg As in soil shows a similar pattern. In spite of this, it is not known what the true exposure concentrations were and the results cannot be extrapolated to the field. The reports of both sets of authors had the same short comings regarding chemical analysis and the overall description of the methodology.

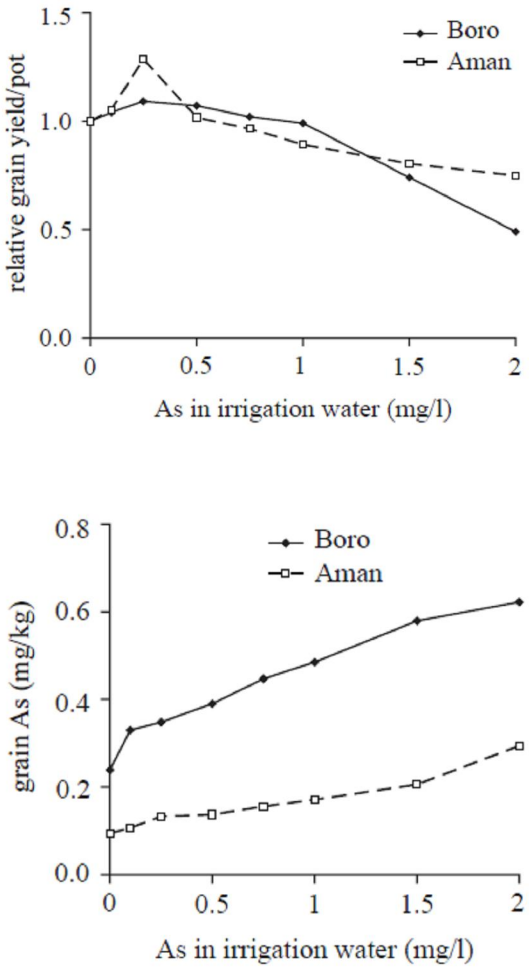

Figure 3. The effect of As on grain yield and on As concentrations in grains of Boro and Aman rice cultivars consecutively grown in the same pots.

Note: Pots were irrigated with contaminated water only during the Boro cultivation. As-free irrigation water was used during the following Aman cultivation.

In conclusion, none of the existing toxicity data can be regarded as representative of the field situation and extrapolations are not yet possible. A better understanding of As in the soil in relation to uptake and toxicity is therefore urgently needed. Ideally, soil parameters should be identified that correlate with uptake and toxicity. The development of a methodology for toxicity experiments that give results representative of field conditions has to be emphasized. With the elevated As levels found in various paddy fields because of long–term irrigation with contaminated water, it may be possible to study phytotoxicity at the field level. Results from such studies would by definition be representative of the field situation, but a thorough understanding of the critical parameters involved would still be necessary in order to extrapolate the data to locations with other environmental conditions (Heikens, 2006).

4. Discussions

There are indications that soil concentrations are increasing over time because of irrigation with As-contaminated water. Data are, however, insufficient in terms of quantity and quality. It is thus still unclear under what specific conditions and over what period of time As is accumulating in the soil. The risk of As-contaminated irrigation water to crop production has received little attention until now. To evaluate current and future soil concentrations, representative toxicity data for crops are needed, both for flooded and non-flooded soil conditions. Thus, field studies to test if As is one of the factors limiting growth in the field should be emphasized. Further, it should become clear what soil parameters correlate with uptake and toxicity and, based on that information, a toxicity database for different rice cultivars and other crops could be developed to set standards for As in flooded and non-flooded soils.

This review has attempted to summarize the incidents of arsenic contamination in the irrigation water-soil-plant system. It poses a significant risk to public health. Therefore, the first priority to remediate the crisis should be early identification of the affected sources, and the next hurdle is to provide arsenic-safe water to the affected masses. It is necessary to seal the highly contaminated tube wells to protect the non-contaminated aquifers.

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Improvement of Oxidation Stability of Mineral Oil using Jojoba Oil

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Abstract: The production of insulating mineral oil from naphthenic fraction (b.r. 300-420°C) was carried out by furfural solvent extraction. The refined oil and its binary mixtures with jojoba oil at different concentrations 20, 50, and 80 vol % have been employed as synthetic insulating oil in a wide variety of electrical equipment. The physico-chemical properties of the refined oil as well as the electrical properties of the mixtures were determined. The oxidation stability of original oil, refined mineral oil and its binary mixtures with jojoba oil with different concentrations was studied. The stability of oxidation by adding different concentrations of 2,6,-di-tertiarybutyl phenol inhibitor to binary mixture containing 20 vol % jojoba oil was studied. It is found that the maximum stability is obtained by adding 2 wt % of inhibitor.

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Key Words: Mineral oils, Oxidation stability, Jojoba oil, Inhibitor, Electrical properties

1. Introduction:

Insulating oils should have stable high-quality properties, not only in the original state, but also during the up time in operation. The oxidation stability of insulating oils has an elementary meaning during operation, because they work under high temperatures usually in the presence of oxygen, so they should resist oxidation.

The oxidation of oil increases its acidity and the content of sediments. Low sediment values indicate high oxidation stability, leading to long oil life. Minimizing the creation of sediments, the dielectric dissipation factor, corrosion of metals,
electric failures maximize the insulating stability of oil[1].

Oxidation stability is an indicator that allows us to set stricter limits for oils in special applications. In some countries, striker limits or other requirements and tests are imposed [2].

In order to settle down the environmental and sustainable issues, people started to look for alternative sources for insulating oil. The latest insulating oil implementation is vegetable oil-based fluid which is known as the most potential source to replace the mineral oil because of its biodegradability characteristic. The first vegetable oil was used for capacitor insulation in 1962 and gave a good match with cellulose due to its higher dielectric constant [3,4].

Jojoba oil has potential as a substitute for some of the petroleum-derived products. It is observed that jojoba oil as a component enhances and also imparts certain properties to the base oil which otherwise can only be realized by doping with additives, thereby helping partially to substitute mineral oil base stocks and to reduce or eliminate the use of some of the additives.

Pure Natural Jojoba is structurally and functionally much different than any other botanical product. Its unique array of pure mono unsaturated liquid wax esters, consisting of long chains of fatty acids and alcohols is very unlike the large branched triglyceride molecules of all other seed oils. The extraordinary oxidative stability and non-occlusive moisture control of jojoba esters provides a highly safe [5, 6]. Jojoba oil has good lubricity and can be utilized as a component. Jojoba oil is an ester of fatty alcohols and fatty acid as shown in its molecular structure [17]

$$CH_3\ (CH_2)_6\ CH_2CH{=}CH\text{-}CH_2(CH_2)n - C(=O) - O - CH_2\ (CH)m - CH_3$$

Where ,
n= 7,9,11 and m = 6,8,10,12

The physicochemical properties of the oil when compared with those of mineral base stocks showed that the pour point, acid value and oxidative stability were the limiting factors in its use as a base stock [7].

Jojoba oil is unique because it is wax ester instead of a typical triglyceride. Jojoba wax esters are similar to those in sperm whole oil and can be utilized in areas, where sperm whole oil has been used in the past. Jojoba liquid wax is stable lipophilic, nontoxic oil obtained frm the desert plant jojoba. This liquid wax differs from common vegetable oils and animal fats in its composition mainly of linear wax esters (97 %).Jojoba being stable to oxidation, may remain chemically

unchanged for years. The wax and its derivative have potential commercial uses in a variety of fields including pharmaceuticals and lubrication [8 - 10].

Mineral oil insulating fluids undergo oxidative degradation in the presence of oxygen to give a number of oxidation products. The final products of oxidation are acidic materials that can affect the characteristics of the insulating fluid as well as cause damage to the components of the electrical unit. Oxygen is a di-radical species and the reactions of the oxidative process are complex but they do involve free radical reactions. One way to prevent these types of reaction is to incorporate an oxidation inhibitor that will interrupt and terminate the free radical process of oxidation. Phenolic materials are quite good for this purpose and the two most commonly used inhibitors are 2,6-ditertiary-butylphenol (DBP) and 2,6-di-tertiary-butyl-4-methylphenol or 2,6-di-tertiary-butyl-paracresol (DBPC) [11-13].

This paper deals with the study of oxidation stability of a naphthenic mineral oil, its rafinate obtained from furfural extraction with solvent/oil ratio 4:1 and binary mixtures of jojoba oil.

2. Experimental:

1- Preparation of the tested oil sample.

The naphthenic acid fraction from suez Co. b.r. 300 – 420^{o}C was refined by using furfural extraction process to prepare raffinate of varying quality. A glass double jacketed mixer settler unit having 0.5 litre capacity was used. The oil fraction and furfural solvent mixed at ratio 4: 1vol was stirred for a period of time (one hour) at 70 ^{o}C then settled for the same time before separate the two phases.

The solvent was removed from raffinate phase by washing with distilled water and dried over anhydrous calcium chloride.

The raffinate oil obtained was mixed with Jojoba oil at different concentrations 20, 50 and 80 vol%. These mixtures were evaluated for improving the characteristics of the mineral oil for insulating performance.

2- Physico–chemical properties Determination

The physico–chemical properties of the naphthenic fraction, its refined oil and its binary mixtures with Jojoba oil were determined according to the standard methods in IP [14] and ASTM [15].

The electrical properties power factor (tan δ) for the previous samples were measured using “HIO KI 3532 Z high Tester” [16].

The naphthenic oil fraction and its refined oil were separated into their hydrocarbon components by using silica gel column chromatography [15].

Oxidation Test:

The oxidation stability of the refined oil produced by furfural extraction and its binary mixtures with jojoba oil with concentration 20, 50, and 80 vol %, were tested according to the oxidation test method ASTM D-1313 and IP-307[15].

The tested samples (40ml for each) were oxidized at 120^{o}C using copper coil as a catalyst. Pure gaseous oxygen (purity 99.9%) flow was adjusted to 2.5 liter/hrs. For the analytical program and the oxidation test was continued up to 210 hrs.

The rate of oxidation was measured by the increase in total acid number, sludge formation and change in power factor.

The binary mixtures of refined oil containing 20 vol.% of Jojoba oil were oxidized using various concentrations of 2,6-Ditert-butyl-phenol-0.5 wt% - 2wt%).

3. Results and Discussion:

The physico-chemical properties of the naphthenic mineral oil fraction and the rafinate used in this work are shown in table (1).Table (1) shows that the tested oil fraction are characterized by pour point (21 ^{o}C), density (0.8958 mg/cm^{3}), below viscosity (1327 cSt), viscosity index(111.2), refrective index(1.4877), viscosity garvity constant(0.45), high aromatic content (50wt%), high sulfur content(2.20wt%), nitogen content(0.123wt%) and total acid number(0.05 mgKOH/g) sample.

Table (1) shows that refining process causes a decrease in physical constants. It is clear that the viscosity of refined oil does not exceed the limits of standard specifications. The viscosity index (V.I.) increases from (111.2) for naphthenic oil to (140.5) for the rafinate. The total acid number of the refined oil decreased after solvent refining to zero value , giving good oil.

The precentage of the removal of aromatics content is 50%, of sulfur content is 63wt% and nitrogen content is 80.48%.The Refining process decreases mostly the aromatic hydrocarbons in the form of di-and polycyclic aromatics while the monocyclic aromatics are not affected to a big extent as given in Table (1). It is observed that the polycyclic aromatics are completely removed. The refining process removes 80% of dicyclic aromatics.

Table (1): Physico-chemical properties of refined mineral oil sample obtained from Suez fraction (b.r.300-420°C) by furfural extraction process:

Physical properties	Method	Origin	Furfural raffinate 4:1
Yield,wt%		-	50.9
Density,g/L,15.56°C	IP-190	0.8958	0.8510
Refractive index,70°C	ASTM D-1747	1.4877	1.4696
Pour point,°C	ASTM D-97	21	30
Mean Molecular weight		228	350
T.A.N.,mg KOH/g	ASTM D-664	0.05	nil
Sulfur content,wt%	ASTM D-	2.2	0.8
Nitrogen content, wt%	ASTM D-	0.123	0.024
Kinematic viscosity, cSt @40 @100 Viscosity index, V.I.	ASTM D-445	 13.27 3.24 111.2	 11.27 3.09 140.5
Flash point,°C	ASTM D- 92	180	188
Hydrocarbon components			
Saturates, wt%		60.00	81.8
Monocyclic aromatics, wt%		14.0	15.2
Dicyclic aromatics, wt%		16.0	3.2
Polycyclic aromatics, wt%		10.0	0.0

Oxidation Stability:

The data presented in Table 2 and Figs. (1-3) illustrate the oxidation stability of the original oil, refined oil and its binary mixtures with different volume percent of Jojoba oil. It is found that the rate of increase in total acid number, sludge formation and tan δ is small in the initial stage of oxidation till (140hr).The reverse occurs at the advanced stage of oxidation. This is due to the fact that the accelerating effect of the oxidation products at this period.

Table (2) and Figs. (1-3) show that the oxidation stability of the original sample is lower than that of the refined mineral oil which is detected from the high sludge formation (1.03-3.0 wt%) as well as the increase in total acid number (0.38-1.17 mg KOH/gm) for the original oil as compared with that of the refined oil where sludge increases from (0.00035-0.0025 wt%) and T.A.N. increases (0.39-0.96 mgKOH/gm) at all times of oxidation process. This due to the presence of high percentage of aromatics, sulfur and nitrogen contents which effect the oxidation rate of the original oil as compared with that of the refined oil as shown in Table (1). The effect of mixing of refined oil with different volume percent of Jojoba oil (20, 50 and 80 vol%) has been studied. The data in Table (2) indicate that blending of Jojoba oil improves the oxidation stability of refined oil to some extent and improve tan δ. The oxidation stability reaches a maximum value by adding 80 vol.% of Jojoba oil. This may be attributed to the high stability of Jojoba oil.

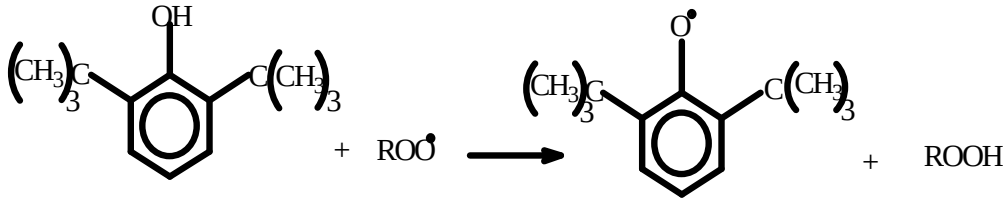
The results in Table (2) indicate that the power factor (tan δ) rises slowly with oxidation time. The value of tans δ of the original oil is higher than that of refined oil. It increases by time (0.62-0.78) for original oil while reaches to 0.67 for refined oil. This is due to the fact that the solvent refining removes the polycyclic aromatic which contain some type of sulfur and nitrogen compounds which effect tan δ. The power factor values of binary mixtures show lower values by oxidation due to the high insulating properties of Jojoba oil and high its stability.

Table (2): Effect of oxidation on the properties of Suez oil fraction, Refined Mineral oil, Pure Jojoba Oil, and its binary mixtures with Refined Oil.

Tested sample	Zero	70 hours			140 hours			210 hours		
	T.A.N.	T.A.N.	Sludge	tan δ	T.A.N.	Sludge	tan δ	T.A.N.	Sludge	tan δ
Original	0.05	0.38	1.03	0.62	0.72	2.01	0.67	1.17	3.0	0.78
Refined Mineral Oil	Nil	0.39	0.00035	0.61	0.48	0.001	0.63	0.96	0.0025	0.67
Pure Jojoba Oil	0.07	0.11	Nil	0.48	0.15	Nil	0.54	0.23	0.0001	0.57
20% Jojoba Oil	0.02	0.21	Nil	0.43	0.34	0.001	0.45	0.71	0.0014	0.51
50% Jojoba Oil	0.04	0.13	Nil	0.72	0.24	0.0004	0.30	0.52	0.0012	0.51
80% Jojoba Oil	0.06	0.08	Nil	0.7	0.19	Nil	0.9	0.34	0.0001	0.26

Effect of Inhibitor on the Oxidation Stability:

An inhibitor material that has found almost universal approval is known chemically as 2,6-Di-tert-butyl phenol was used. This material is very desirable inhibitor and has outstanding properties which even in small concentrations are stable and effective as oil antioxidant. A concentrations ranging from (0.5- 2 wt%) of inhibitor were used with the original oil, refined oil and its binary mixture (20 vol%) with Jojoba oil tt reduces the alkylperoxy radicals and alkyl hydroperoxide as observed by the following equation:



The rate of oxidation as shown in table (3) and Figs. (1-3) decreased in all samples which is detected from the low values of total acid number for original oil, refined oil and binary mixture with Jojoba respectively, this is due to the termination of the chain reaction of oxidation by the action of hydroxyl group in inhibitor which is suggested to absorb oxidation radicals. The maximum stability is obtained by adding 2% of inhibitor. This means that the effect of inhibitor on stability of oil depends on its concentration and also on the degree of refining.

Table (3): Effect of Inhibitor on the Oxidation stability of Suez oil fraction, Refined Mineral oil, Refined Mineral Oil with 20 vol% Jojoba Oil

Tested sample	T.A.N, mg KOH/g								
	70 hours			140 hours			210 hours		
	0.5%	1%	2%	0.5%	1%	2%	0.5%	1%	2%
Original	0.34	0.28	0.23	0.63	0.54	0.41	0.98	0.83	0.71
Refined Mineral Oil	0.30	0.25	0.17	0.40	0.32	0.26	0.81	0.74	0.60
20% Jojoba Oil	0.18	0.13	0.10	0.30	0.21	0.16	0.61	0.50	0.39

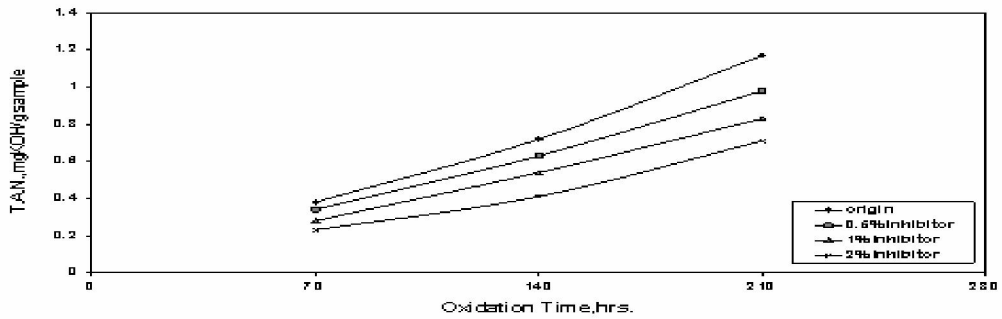


Fig. (1): Effect of DTBPh Inhibitor on the T.A.N. of the Suez Naphthenic Oil Fraction

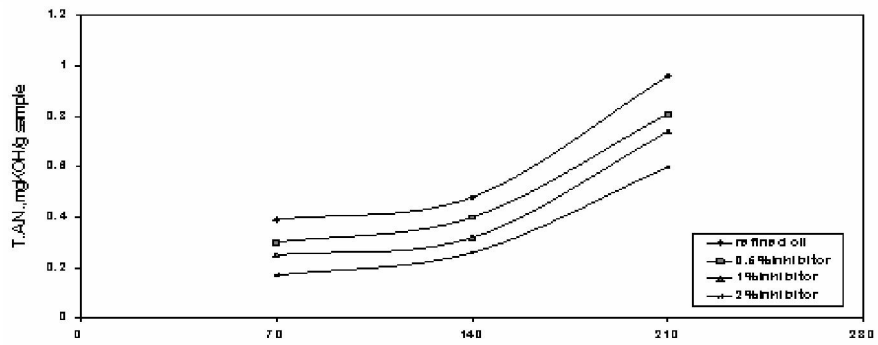


Fig. (2): Effect of DTBPh Inhibitor on the T.A.N. of the Refiend Mineral Oil

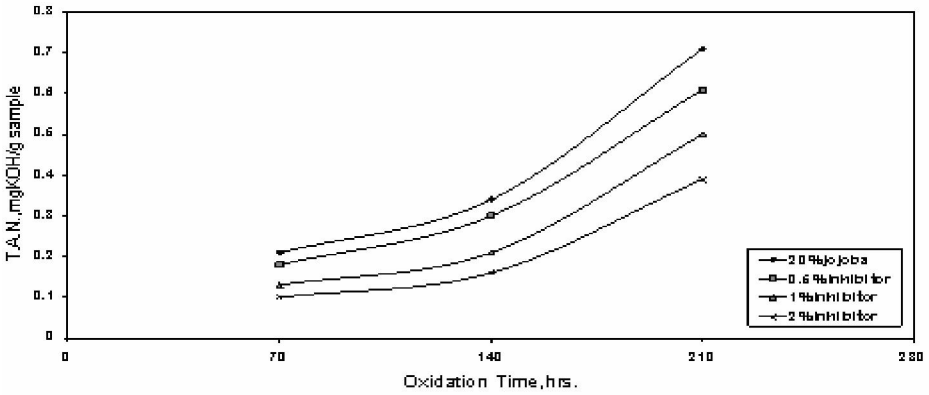


Fig. (3): Effect of DTBPh on T.A.N. of the Refined Mineral oil with 20 Vol % Jojoba Oil.

4. Conclusion:

- The refining processes increase the oxidation stability of the oil.
- The oxidation stability of the binary mixtures is highly improved by increasing the volume percent of jojoba oil.
- The values of power factor (tan δ) for binary mixtures show lower values by oxidation due to high insulating properties of these oils.
- The maximum stability reaches by adding 2 wt % inhibitor to 20 vol % binary mixture of jojoba oil with refined oil.

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Mapping water quality of Burullus Lagoon using remote sensing and geographic information system

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Abstract: The present study aims to utilize remote sensing and a geographic information system (GIS) for mapping surface conditions of the Burullus Lagoon, Egypt as a proxy to water pollution. Spatial distribution of suspended matter, nitrogen, phosphorous, chlorophyll, dissolved oxygen, water temperature, salinity, depth, lead, copper, cadmium, clay, and sediment organic carbon has been applied. A Landsat image from the Enhanced Thematic Mapper plus (ETM+) sensor acquired in June 2006 was processed based on a band by band as well as band rationing. Cartographic maps were generated depending on the correlation between the measured parameters and the radiance values of the ETM+ image. Parameters not correlated with the satellite image data have been processed through spatial analysis and interpolation technique using GIS. Results showed that the eastern and southern sections of the lagoon, which receive drainage wastewater, are more polluted than the northern and western sections of the lagoon. The study confirms that remote sensing coupled with GIS could afford an integrated scheme for mapping water quality.

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Keywords: Mapping; water quality; Burullus Lagoon; geographic information system

INTRODUCTION

Burullus is one of four shallow coastal lagoons at the Nile Delta of Egypt; namely: Manzala, Burullus, Edku and Maryout (Fig. 1). Burullus lagoon is located between the two Nile branches Rosetta to the west and Damietta to the east. It extends between longitudes 30°31` and 31°05` E and latitudes 31°25` and 31°35`. It occupies an area of 420 km² of which 370 km² are open water (Guirguis et al. 1996). The rest of the area comprises a group of islands distributed within the water body. The length of the lagoon is about 53 km, its width is about 13 km and it has water depths ranging from 0.5 to 2.5 m (Frihy and Dewidar, 1993). It connects to the sea through a narrow strait called Al-burg inlet or Boughaz El-Burullus at its northeast side. The lagoon is separated from the sea by a narrow coastal strip covered by sand sheets and sand dunes. The Burullus lagoon receives drainage water at its southern boundary through seven drains. It also receives fresh water from Brimbal Canal situated at its southwestern corner (Okbah and Hussein, 2006). Agricultural lands encompass the southern and eastern fringes of the lagoon. The environment of Burullus Lagoon has witnessed the significant change during the last three decades as many drains were constructed to convey agricultural wastes into the lagoon. In addition, substantial area has been dried up to agricultural land.

Remote sensing, which is the science of obtaining information about an object without being in physical contact, has been widely used with GIS in water quality studies (Usali and Ismail, 2010). Generally, turbidity, chlorophyll and nutrients can change the reflected or emitted electromagnetic radiation from surface water. These changes could be monitored by remote sensing. Empirical relationships between spectral properties and water quality parameters were established as early as 1970s (Morel and Gordon, 1980). Substances that do not affect the spectral properties of surface water could be mapped by other modeling surrogates (Ritchie and Schiebe, 2000). GIS is one of these techniques.

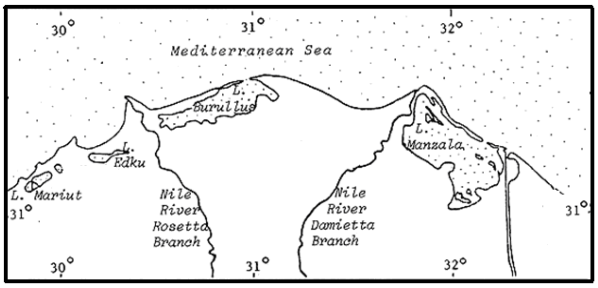

Figure (1): Location map of the Nile Delta showing the four coastal lagoons.

The ecology of Burullus Lagoon has been studied by El-Sherif (1993); Aboul Ezz (1995); Khedr (1999); Ramadani et al. (2001); and Radwan (2002). Bottom sediments and heavy metal pollution studies were carried out by El-Sabrouti (1984); El-Sammak and El-Sabrouti (1995); Abdel-Moati and El-Sammak

(1997); Radwan and Lotfy (2000) and Abdel-Baky et al. (1998). Remote sensing is a new technique used to map water quality parameters, such as chlorophyll content and suspended matter (Arenz et al. 1996; Dewidar and Khedr, 2001 and Vincent et al., 2004). This technique can be applied for lakes and reservoirs (Lathrop and Lillesand 1989), and tropical coastal areas (Ruiz- zuara, 1995). Dewidar and Khedr (2005) utilized Thematic Mapper (TM) data combined with field data measurements during April 2004 to map the depth, salinity, sand, and sediment organic matter in the Burullus Lagoon. The major objective of the present study is to map water pollution in Burullus Lagoon using chemical analysis, remote sensing and GIS.

MATERIALS AND METHODS

a) Water and sediment analysis

Eighteen sites covering the entire Burullus Lagoon body were identified during May 2008, and each site was represented by a surface water sample and a bottom sediment sample. Sampling locations were grouped into three regions: east (6 samples), middle (7 samples) and west (5 samples) of the lake (Fig. 2). Water samples were taken by a cleaned one liter polyethylene bottle immersed 20 cm below the water surface. At each site, field measurements were carried out; including: 1- the geographic location using a Garmin Map GPS; 2- water depth using a pocket echo sounder; 3- water salinity using a portable TDS meter and 4- water temperature. In the laboratory, the following parameters were determined in water: 1- turbidity using the standard nephelometric method (Environmental Protection Agency, EPA, 1983); 2- dissolved oxygen using the standard Winkler method (American Public Health Association, APHA, 1985); 3- chlorophyll (a) according to APHA (1985); 4- ammonia-nitrogen according to APHA (1989); 5- dissolved reactive phosphorous (DRP) by the direct stannous chloride method (APHA, 1985) and 6- the heavy metals (Cd, Cu and Pb) by the solvent extraction method (APHA, 1992). The sediment samples were collected by a grab sampler. The samples were air dried and the following parameters were measured: 1- the grain size analysis using the Hydrometer methods (piper, 1955) and 2- the organic carbon content using the Walkley and Black rapid titration method (Black, 1965).

b) Remote sensing analysis

A Landsat ETM+ image acquired in 12 June 2006 (Path 177 and Row 38) was used in this study as this date was the most available for the analysis. This image contains 7 bands (three in the visible and four in the infrared portions of the spectrum). The thermal band was eliminated from the ETM+ image and the image processing was applied to the other 1-5 and the 7^{th} bands. The image was registered to the Universal Transverse Mercator (UTM) Projection using several well distributed ground control points (GCPs) obtained from 1:50,000 topographic maps. A subset image covering the boundaries of the Burullus Lagoon was created. At this subset image, the raw digital numbers were converted to radiance value. Correlation matrices were used to explore the relationship between the measured water quality parameter and the ETM+ radiance data. Regression relationships were generated between the individual band readings and the water parameters as well as between band ratios (b1/b2, b1/b3, b1/b4, b2/b1, b2/b3, b3/b1, b3/b2, b3/b4, b4/b2, b4/b3 and b7/b5) and the water parameters. Statistical analysis was carried out using SYSTAT software (Wilkinson, 1997). Image processing techniques were performed using ERDAS Imagine software. Cartographic maps of the correlated parameters were created in ArcMap software.

c) GIS analysis

The GIS analysis was carried out using ArcGIS software in order to make spatial distribution maps of the parameters not correlated with the satellite data. The boundary of the lake was digitized using the polygon shapefile. The geographic locations (Longitudes and Latitudes) of the sampled sites were inserted as a basic separate layer and a database table containing the results of the different water and soil parameters was created. For each parameter, the spatial analysis was applied based on the interpolation and surface analysis methods yielding a contour map. Then a clip image containing the classified spatial distribution map for each measured water and sediment parameter was extracted.

RESULTS

The correlation matrix between the examined water and sediment parameters and the radiance values of the satellite image data is shown in Table (1). Only turbidity, chlorophyll-a, temperature and ammonia nitrogen are correlated with the radiance values of the ETM+ image. Turbidity is highly positively correlated with B4/B2 ($P<0.01$) and B4/B3 ($P<0.05$); chlorophyll-a is highly positively correlated with B4/B2, B4/B3 ($P<0.001$) and B7, B5 ($P<0.01$); temperature is highly positively correlated with B3/B1 ($P<0.01$) and B2/B1 ($P<0.05$); and ammonia nitrogen is positively correlated with B2, B3 and B2/B3 ($P<0.05$). The models representing the statistical relationships for the measured water parameters and the radiance value of the ETM+ satellite image are as follows:

Table (1): Correlation matrix between the environmental variables and the ETM+ data for Burullus Lake. * P<0.05, ** P<0.01, *** P<0.001.

$N\text{-}NH_3$ ppm	DRP ppm	Pb ppm	Cu ppm	Cd ppm	Chlo (a) mg/l	Turb. NTU	TDS, ppm	D.O., mg/l	Temp, °C	Depth, m	
0.300	0.0855	-0.204	0.111	0.429*	0.0333	-0.0431	-0.0859	-0.206	-0.633***	0.440*	B1
0.382*	0.0137	-0.0484	0.190	0.361*	-0.0858	-0.208	0.204	-0.128	-0.443*	0.200	B2
0.363*	0.0271	-0.214	0.186	0.489**	0.0712	0.0182	0.0671	-0.283	-0.406*	0.315	B3
0.164	-0.184	-0.323	-0.209	-0.0174	0.299	0.0684	-0.223	0.240	-0.631***	0.354*	B4
-0.0025	0.149	-0.333	0.258	0.453**	0.551**	0.346	-0.187	-0.202	0.0634	0.214	B5
0.0611	0.169	-0.389	0.276	0.520**	0.516**	0.335	-0.132	-0.279	-0.011	0.280	B7
0.335	0.0947	-0.144	0.152	0.447*	0.00143	-0.0814	0.0222	-0.205	-0.532**	0.364*	B1/B2
0.311	0.113	-0.187	0.136	0.465**	0.0474	-0.00937	-0.00128	-0.246	-0.512**	0.383*	B1/B3
0.0885	0.313	0.0391	0.345*	0.652***	0.250	0.249	-0.0637	-0.287*	0.176	0.122	B1/B4
-0.0258	-0.277	0.0962	-0.0184	-0.422*	-0.173	-0.243	0.240	0.283*	0.450*	-0.520**	B2/B1
0.354*	-0.0404	0.215	0.169	0.131	-0.296	-0.506**	0.365*	0.132	-0.382*	0.0129	B2/B3
-0.0565	-0.238	-0.0659	-0.0295	-0.320	-0.0209	-0.0165	0.186	0.124	0.531**	-0.465*	B3/B1
0.262	0.122	-0.370*	0.152	0.564***	0.251	0.285	-0.0549	-0.437*	-0.271	0.390*	B3/B2
0.205	-0.0768	-0.0336	0.0362	0.0269	-0.418*	-0.236	0.302	-0.225	-0.286	-0.0039	B3/B4
-0.132	0.102	-0.155	0.104	0.281	0.648***	0.462**	-0.257	0.0609	0.155	0.132	B4/B2
-0.120	0.0732	-0.111	0.109	0.252	0.626***	0.413*	-0.253	0.115	0.161	0.094	B4/B3
0.239	0.0585	-0.461*	0.255	0.518**	0.326	0.197	0.0319	-0.344*	-0.185	0.259	B7/B5

TEMPERATURE MODEL:

$Y_{temperature} = a + bX1 + cX2 + dX3$

Where $Y_{temperature}$ = Temperature expressed in degrees centigrade.

X1 = B1,

X2 = B4,

X3 = B1/B3, and

a = 21.747, b = - 276.678, c = - 1.538, and d = 20.776

TURBIDITY MODEL:

$Y_{turbidity} = a + bX1 + cX2 + dX3$

Where $Y_{turbidity}$ = Turbidity expressed in NTU.

X1 = B2/B3,

X2 = B4/B2,

X3 = B4/B3, and

a = 100.409, b = - 68.853, c = 213.145, and d = - 187.046

CHLOROPHYLL (a) MODEL:

$Y_{chlo.(a)} = a + bX1 + cX2 + dX3$

Where $Y_{chlo.(a)}$ = Chlorophyll (a) expressed in milligram per litter.

X1 = PC1,

X2 = B3/B4,

X3 = B4/B2, and

a = -8.145, b = 68.036, c = 25.986, and d = 44.195

AMMONIA-N MODEL:

$Y_{Ammonia\text{-}N} = a + bX1 + cX2 + dX3$

Where $Y_{Ammonia\text{-}N}$ = Ammonia-N expressed in milligram per litter.

X1 = B2,

X2 = B3,

X3 = B2/B3, and

a = -100.988, b = -1911.345, c = 1486.719, and d = 273.861

Cartographic maps for the distribution of turbidity, chlorophyll, temperature and nitrogen based on these models are shown in Figure (2). These maps show that the chlorophyll concentration is higher at the

southern part of the lagoon than the other sides and the lagoon has low water turbidity for its major basins. In addition, the nitrogen distribution coincides with the chlorophyll distribution and the water temperature is homogeneous throughout the water body (22-24°C) except some minor colder basins (20-22°C). Other parameters (depth, dissolved oxygen, salinity, phosphorous, Pb, Cd, Cu, OC, and clay content) did not have correlation with the image radiance values. Consequently these other parameters were spatially distributed using the interpolation method in ArcGIS (Fig. 3).

Figure (3) shows that the lake is generally shallow (<140 cm). The eastern side is shallower than the rest of the lake. The deepest region of the lake occurs at the middle basin (> 140 cm). Water salinity is generally lower at its western sector and the dissolved oxygen of the majority of the lake basins approaches 5 mg/l. Phosphorous is maximal in the middle part of the lake. The distribution of the clay and organ carbon is generally correlated, where most of the lake sediments range in clay content between 20-30% and in the organic matter between 2-3%. The heavy metals' distribution indicates that lead is lower at the northern part of the lake, whereas copper has a heterogeneous distribution. Cadmium generally increases westward.

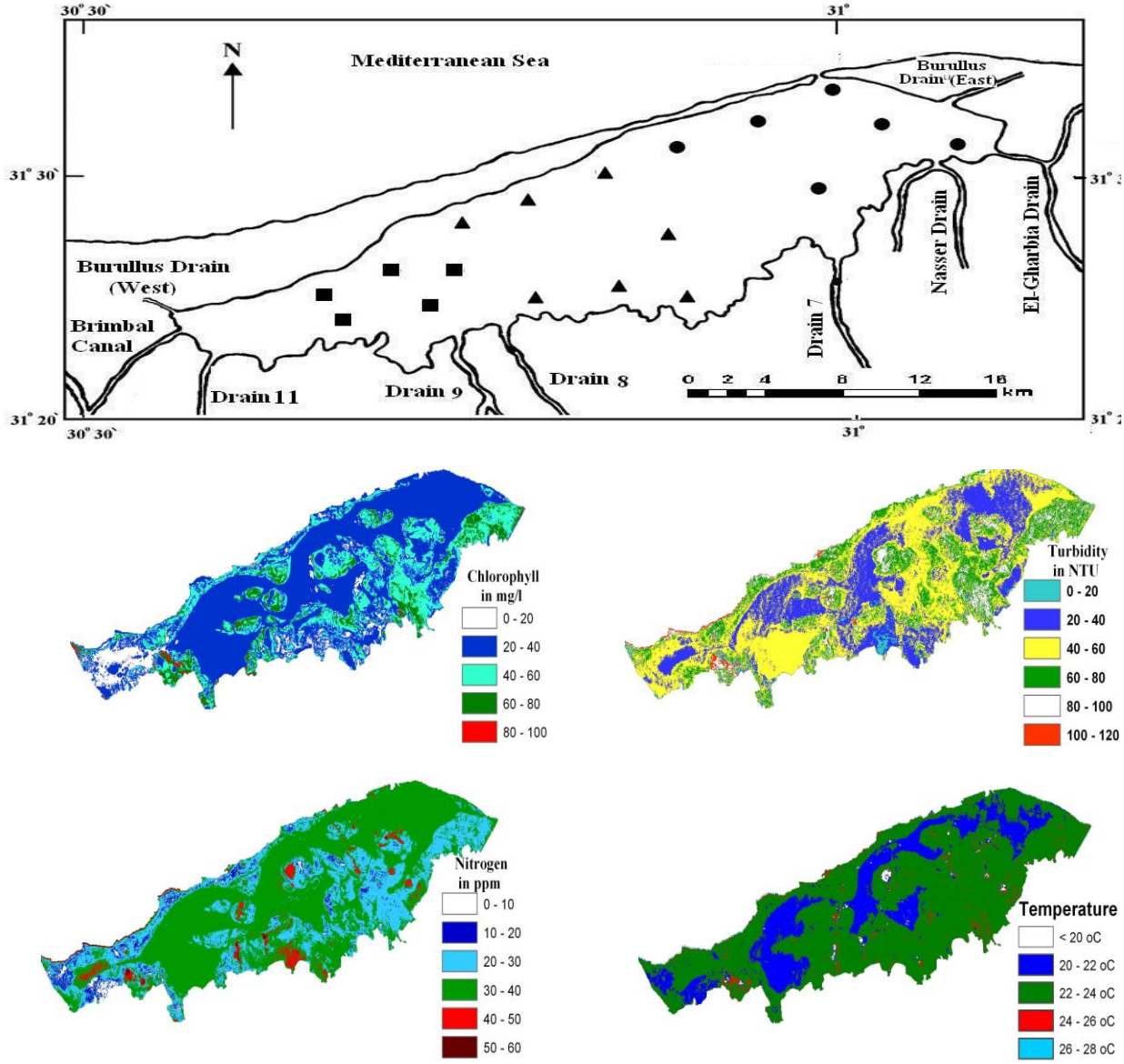

Figure (2): Sampling sites and cartographic maps of chlorophyll, turbidity, nitrogen and temperature distribution as obtained from remote sensing analysis.

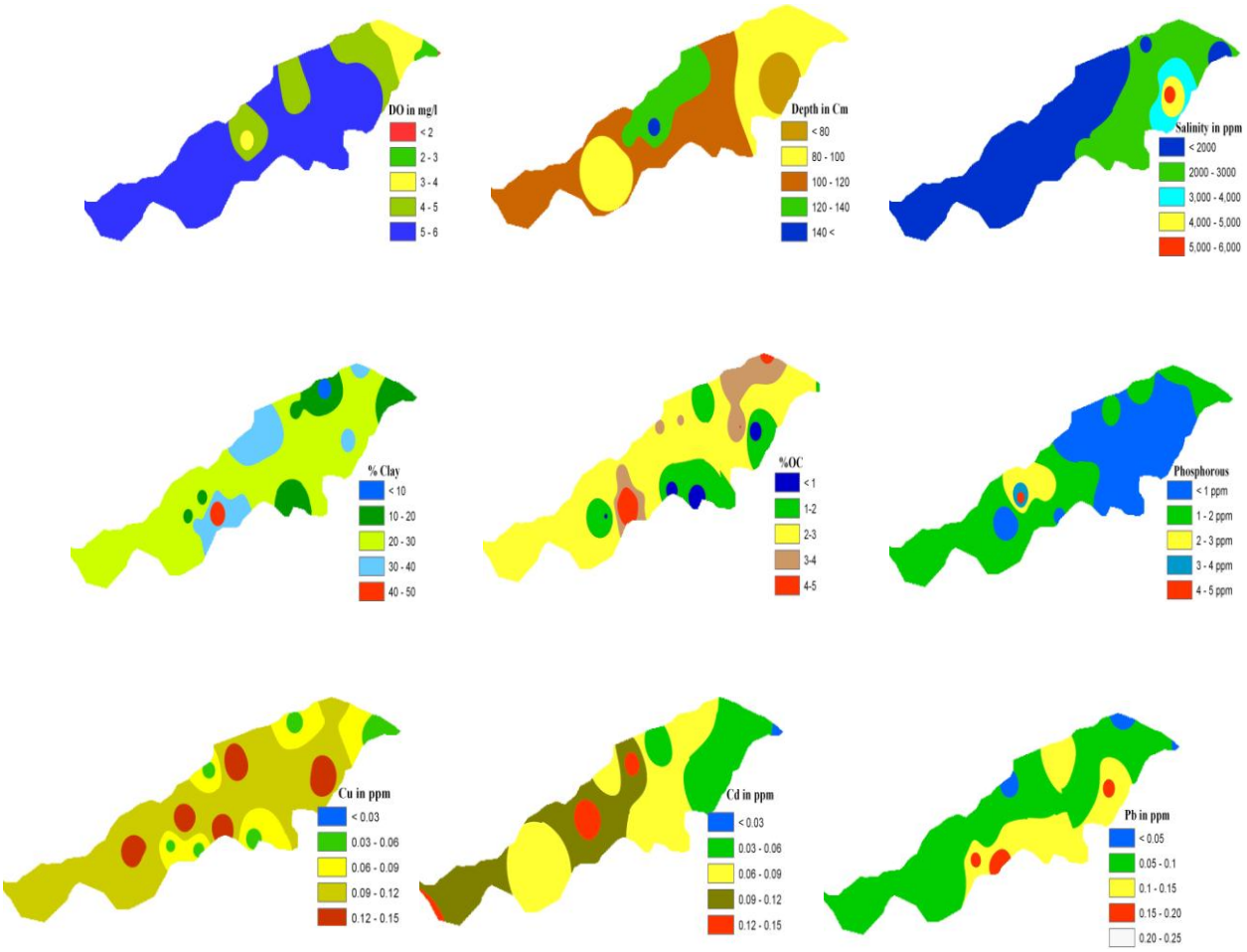

Figure (3): Cartographic maps of salinity, depth, DO, phosphorous, OC%, %clay, Pb, Cu, and Cd as obtained from the GIS analysis.

DISCUSSION AND CONCLUSIONS

The environmental conditions of the Burullus Lagoon have been impacted by the discharge of agricultural wastes into the water body. Although the lake receives seawater from a narrow straight at its northeastern section, continuous release of wastewater from the southern drains is much greater than seawater discharge into the lagoon. This situation has eventually accelerated eutrophication of the water body due to the increased nutrient inflow to the lake. Figures 2&3 highlight the significant increase of turbidity, nitrogen, and chlorophyll at the southern side of the lagoon, which receive the drainage water from six agricultural drains (Drain 11; Drain 9; Drain 8; Drain 7; Nasser Drain; and El-Gharbia Drain). Phosphorous concentration is observed to be higher in the middle part of the lagoon, which confirms with Okbah (2005). Water salinity of the lagoon is lower in the western section of the lagoon (< 2000 ppm) than in the eastern side. This variation of salinity is attributed to the difference of water salinity of the source water coming into the lagoon. The western section receives fresh Nile water from Brimbal Canal, which dilutes water within the lagoon to less than 2000 ppm. The eastern side of the lake receives water directly from the sea and therefore, water in this side is much salty. Moreover, the chlorophyll content is also minimal at the western side (Fig. 2) due to the discharge of fresh Nile water into this side through Brimbal Canal.

The dissolved oxygen of the lagoon is generally high (5-6 mg/l), particularly at the western side. The easternmost section of the lagoon, which receives the greatest wastewater effluents from four drains (Drain 7; Nasser Drain; El-Gharbia Drain; and Burullus Drain West) containing sewage and agricultural waste, exhibits the minimal dissolved oxygen content. The concentrations of the heavy metals lead, copper and cadmium ranged between (0.03 –

0.17); (0.05 – 0.15); and (0.03 – 0.17 ppm), respectively. The distribution of lead is greater in the southeastern section. Copper is released into water from the boat paintings of local fishermen. Cadmium is discharged with wastewater, and it is observed greater in the western section than in the eastern side. According to the United States Environmental Protection Agency (USEPA, 1986), both lead and cadmium concentrations are greater than the permissible limits of heavy metals in saltwater.

Bottom sediments of the lagoon have mainly a silty to clayey texture, where the majority of lake sediments contain 20-30% clay. Organic matter distribution coincides with clay distribution. Generally, the organic carbon of the lagoon is high, averaging 2-3% of the bottom sediments. These results agree with a previous study by Coutlier and Stanly (1987) who observed that the bottom sediments of the lagoon are mainly silty clay high in organic matter content. In a conclusion, the eastern and southern sides of the Burullus Lagoon are much polluted than the northern and western sides. The primary reason is the occurrence of many drains carrying sewage and agricultural waste at these sides.

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Path Analysis of Direct and Indirect Effect of Statistical literacy on Applying Proper Statistical Test (Case Study of agricultural extension and education graduated students)

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Abstract: Research methods, statistical analysis and domination on subject are essential for a rich dissertation and thesis to be developed. The main goal of this study was to obtain the perception of the agricultural extension and education graduated students about their statistical literacy, reasoning and thinking according to standard tests and to trace thematic evolution (content analysis) of dissertations and thesis done by the same graduated students according to sequential statistics analysis approach (SSAA). To this end, the study analyzed 315 thesis and dissertation to understand, how and to what extent, proper and mix statistical methods are applied to achieve realistic outcomes. In the other hand, 115 questionnaires were fulfilled, containing statistical standard tests about statistical literacy, reasoning, thinking, attitude, content knowledge and principal component of statistics learning. According to the path analysis results, the statistical attitude (total effect=0.80) had the most effect (direct and indirect effect) on applying statistical methods.

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Keywords: statistical literacy, statistical reasoning, statistical thinking, sequential statistical analysis approach

1. Introduction

The growth of agriculture and nature resources as scientific disciplines especially agricultural extension, education and development that have the maximum accordance to social and behavioral sciences depend on many variables (Dyre et al 2003). Flyvbjerg (2001) argued many researches were driven by a continuing belief that the social and political world could be measured through objective, empirically testable and law-like data indicators.

Fincher (1991) stressed that "research on the substantive issues is handicapped by higher education, s lack of status and recognition as an academic discipline and or professional specialty", the issue of understanding research as a disciplined inquiry is still stealth, especially in higher education. Likewise, Dijkum (2001) indicated that analysis of the practice of social research shows there is no easy answer to the question of how the knowledge of the natural sciences can be used to further understanding in the social sciences. It is useful to know that the inseparable section of scientific inquiry is statistical analysis and one of the important sections of statistics is using tests to analyze data. To obtain appropriate data, knowing and dominating on the superior plan or approach is necessary, so using mix and sequential proper statistical tests to realize accurate outcomes help researchers. Accordingly, there are more expectations from thesis and dissertation as an academic inquiry.

As indicated by Windish and Diener-West (2006) and Govindrajulue (2004), there are a few, if any, references for using sequential statistics in the literature. Although choosing the right statistical tests for a particular set of data appears to be an overwhelming task. According to Wheater and Cook (2000) particularly, if such decisions are rendered after data are collected, the sequences and the placements of statistical tests to understand their role and mission are in the first place. Wheater and cook (2000) believe that an investigator is definitely responsible for choosing statistical methods. Therefore, she/he must be able to use statistics effectively to organize, evaluate, and analyze the data (Whitney, 2005). To ease the dilemma, it is helpful to identify the level of statistical literacy, reasoning and thinking of researcher. Also it is important to know if the student as a researcher has selected the statistical tests mix or general and if she/he has holistic view about statistical methods election or not? On the other hand, use of statistics as an analysis process in an especially operational research has a vital role to obtain a relatively correct response; however it makes a statistics anxiety (Zeidner, 1991). Eventually, this paper wants to know if usage of sequential statistical analysis approach (SSAA) can help to solve the statistical anxiety and test s' misused among post

graduated students and also if it assists them to develop their statistical literacy, reasoning and thinking ability by meeting their basic needs.

Theory and rationale

Before starting this part, we have to explain the content that will be examined by it and to specify certain theories or perspectives that indicate the content. Statistics has seen as a science of variability and a way to deal with the uncertainty that surrounds us in sciences and our life (Kendall, 1968; Moore, 1997). In particular, statistics is used to describe and to predict the phenomena that require collections of measurements. But what are the essential skills to navigate today's technology and information-laden society?

Students have problems to learn statistics (Meletiou & Lee, 2002). It may be because of some wrong learned statistical concepts and applications. Malek Mohammadi (2009) posed a model in the sequential statistical analysis approach (SSAA) to present a mixed and sequential method. In this model, he mixed three phases and divided each phases to several steps that researchers should use them to refine and improve research; they are as following:

A. Initial phase

1- Variable mining and measurement

-causes associated with the research problem (Cohen et al., 2002),

-kind of research (Moyusky, 1995; Wadsworth, 2005; dinove, 2008),

- Research question (Cohen et al., 2002; Bruin, 2006; Marion, 2004),

-the aim of analysis (Cohen et al., 2002),

-Kind of variable (Wheater&Cook, 2000, MacGraw, 2007; Watt &Vandenberg, 2002; porter, 2001),

-Variable measurement (Kaminsky, 2008; Watt &Vandenberg, 2002; porter, 2001).

2- Variable reduction (refinery)

-Validity and reliability (Ferrando, 2009),

-Coefficient of variability (Malek Mohamadi, 2009),

-Correlation matrix (Malek Mohamadi, 2009).

3- Variables or respondents groping (exploratory factor analysis [EFA])

-Exploratory factor analysis (EFA) (Salkind, 2008).

B. intermediate (inferential phase)

1-Variable and groups identification

-Independent/dependent or endogenous /exogenous variable (Hill&Lewicki, 2007; Kaminsky, 2008),

-Number of groups being compared (Watt &Vandenberg, 2002; porter, 2001),

-Kind of group being compared (Watt &Vandenberg, 2002; porter, 2001)

2- Hypothesis development

-Main hypothesis (Graveter&Forzano, 2008),

3- Hypothesis testing (choosing the appropriate statistical test)

-P value / effect size (Dixon, 2009; Denis, 2003),

-Sample size and complexity of data (McDonald, 2008),

-Central limit theorem (McDonald, 2008; Moyusky, 1995; Wadsworth, 2005),

-Number of independent hypothesis or multiple comparisons

-Paired or unpaired (Moyusky, 1995),

-Parametric/ nonparametric (Moyusky, 1995),

-Choosing the appropriate statistical test (Watt &Vandenberg, 2002; porter, 2001),

C. advanced (modeling) phase\

1- Regression (multiple and multivariable)

2- Structural equation modeling (SEM),

-Path analysis (Salkin, 2008),

-Confirmatory factor analysis (Maroulides, 2006)

Each of the phases is composed of a few steps within which there are general and specific criteria for selecting and applying statistical tests.

To this end, having statistical literacy is a requirement for researchers to understand and use the approach.

Statistical literacy

As more information technology, and world oriented societies, citizens need to sound understand about basic statistics to face with social demands front of them to get consciousness decisions. But what are the basic stats for these people? Probability and Statistics educators to answer this question address people to the statistical literacy.

There are many definitions of statistical literacy. It is defined by Wallman(1993) as "ability to critical evaluation and understand of results that is associated with the ability to be encouraged to participate in statistical thinking that can be in the public and private or professional and personal decisions form."

It is completed by Gal (2002); that required community statistical literacy is composed of two components:

(a) People's ability to interpret and critically evaluate statistical information, data-related arguments, or stochastic phenomena, which they may encounter in diverse contexts, and when relevant

(b) their ability to discuss or communicate their reactions to such statistical information, such as their understanding of the meaning of the information, their opinions about the implications of this information, or their concerns regarding the

acceptability of given conclusions. He also suggested that statistical literacy required making people empowerment to interpret statistical data and critical evaluation, discussion-based data, or random phenomena. Likewise, Watson (2006) sees statistical literacy as the "meeting point of the chance and data curriculum and the everyday world, where encounters involve unrehearsed contexts and spontaneous decision-making based on the ability to apply statistical tools, general contextual knowledge, and critical literacy skills" (Watson, 2006). Chick, Pfannkuch and Watson (2005) describe statistical literacy as 'transnumerative thinking' where students will be able to make sense of and use different representations of data to make sense of the world around them. Gal and Garfield (1997) see statistical literacy as the need for students to be able interpret results from studies and reports and to be able to "pose critical and reflective questions" about those reports because "most students are more likely to be consumers of data than researchers".

Statistical literacy is needed for many reasons: the statistics commonly discussed will be used in the fields of political, economic and social. Students with statistical literacy better are able to use statistical aspects on the social debate to read and interpret. Many students have problem in the areas of statistics discussions. Students have problem with comments and alternative interpretations of statistically events. Students often mistakenly conclude (Chiarella, 2001).

Gal (2002) suggests that statistical literacy involves both knowledge elements and dispositional elements described in the enclosed model. Applied together, they form the basis for statistical literacy. A look at this model shows that statistical literacy is based upon the availability of literacy knowledge and skills, as much as about content knowledge in relevant areas in statistics and mathematics (figure 1).

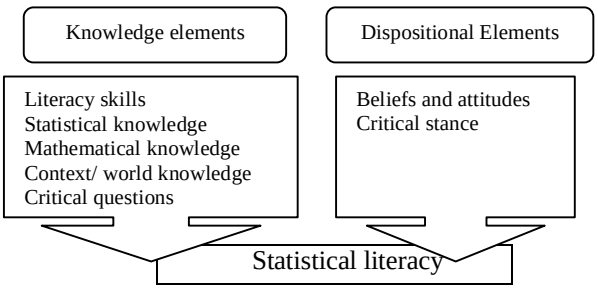

Figure 1- elements of statistical literacy

Meanwhile Statistical Knowledge is a critical component of this model. Clearly, knowledge of statistical and probabilistic concepts and procedures is required for statistical literacy. But since Delams (2002) posed two models that in one of them, statistical literacy was supposed as the all-encompassing goal of instruction and statistical reasoning and thinking no longer have independent content. In this model there are no parts of the domains separate from statistical literacy. In this model statistical reasoning and statistical thinking become "sub-goals within the development of the statistically competent citizen and they have overlapping with together (figure 2).

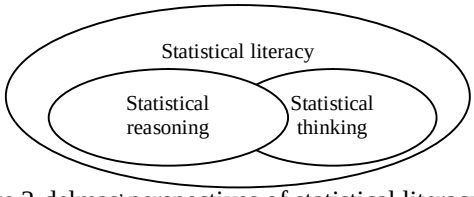

Figure 2-delmas' perspectives of statistical literacy

Statistical thinking

Both Ben-Zvi and Garfield (2004) and Chance (2002) cited Statistical thinking including understanding about why and how reviews are done and "big ideas" as areas for statistical assessment. These ideas include the nature of distribution, when and how to use appropriate methods to analyze data such as summarizing numerical data and visual display of data. Statistical thinking, including understanding the nature of sampling and how researcher can inference from sample for statistical population, and why to create cause and effect relationship is required design experience. This includes understanding how to use the model for simulation a random phenomenon. Also for a review process, how, when and why the existing inductive devices can be used. Statistical thinking also includes being able to understand and difficult field advantage in shaping the review, draw conclusions and to identify and understand the processes has been entered (consider the question of collecting data to analyze selected for testing hypotheses etc). Finally, critical thinkers and statistical evaluation of results of a problem or statistical methods are capable.

Wild and Pfannkuch (1999) five basic types of statistical thinking expressed:

1) recognize the need for Contracts (more than mere attention to the linguistic evidence);
2) trans numeration - ability to get good data that show the real situation to change and provide the data to obtain additional means more of them;
3) Due to Distribution - This means to make judgments about the data is described, including search and dispersal patterns and try to understand in relation to these areas is
4) reasoning about models - from simple (such as tables and charts) to complex; like

those can be found in the pattern, and summarization of data using multiple and

5) Summary statistics - Create relationship between two things that are essential components for statistical thinking.

Elsewhere is that the process of statistical thinking from Groth (2003) perspective is following:

1- Describing Data: The explicit reading of data presented in tables, charts, or graphs.
2- Organizing and Reducing Data: Arranging, categorizing, or consolidating a given set of data into summary form.
3- Representing Data: Displaying a given set of data by using graphs.
4- Analyzing Data: Identifying trends and making inferences or predictions from a data display or set, using formal inferential methods when appropriate.
5- Collecting Data: Planning, conducting, and critiquing surveys, experiments, and observational studies.

Statistical Reasoning

In comparison, statistical reasoning may be defined as the way people reason with statistical ideas to make sense of statistical information (Garfield & Gal, 1999). Ben-Zvi and Garfield (2004) describe statistical reasoning as interpretation of data and its different representations. This includes the interpretation based on those data, the data presented, summarizing the data is. Statistical reasoning may involve a relationship some concepts with another concepts (such as centralization and variability), or may combine ideas about data and probability. Reasoning means understanding and able to explain statistical processes and the ability to interpret statistical results is complete. For example, statistical reasoning about binary variables include to know how to judge the relationship between two variables and interpret and typically include translation or restore processes between rows of data, graphing relations and express the statistical reason of them(Aquilonius,2005). Garfield (2003) describes a five level hierarchy for statistical reasoning that ranges from idiosyncratic reasoning, where students have little or no understanding of words, symbols and concepts, through to integrated process reasoning where students have a complete understanding of a statistical process. Understanding and using statistical language is seen as significant. Sooth Garfield presented a statistical model. He wants that students have greater access to better understanding. His definition of statistical reasoning was the way people argue about statistically ideas and statistically information significance. Understandings of this argue is conceptual understanding of important ideas such as dispersion, centralization, randomize, and sampling (Garfield, 2003).

Interestingly, Garfield suggests that critiquing media reports may be one way to assess statistical reasoning.

The interrelatedness of statistical literacy, statistical reasoning, and statistical thinking does potentially make it difficult for teachers to design lessons or assessments that would meet all the competing goals. DelMas (2002) makes a final attempt to describe the features of statistical literacy, statistical reasoning, and statistical thinking by focusing not on the context or content of

Problems but what teachers ask students to do with the context or content. DelMas outlines in Figure 3assessment questions that are asked in tasks for statistical literacy, reasoning and thinking that would involve students being in “one domain more so than in another” (DelMas, 2002).

Basic literacy	Reasoning	Thinking
Identify Describe Rephrase Translate Interpret Read	Why? How? Explain (the process)	Apply Critique Evaluate Generalize

Figure 3-Three instructional domains

Again it appears here that DelMas (2002) is attempting to limit statistical literacy to a procedural literacy. Statistical reasoning appears to be the ‘doing’ of statistics and statistical thinking the ‘questioning’. While this may be attractive as a description we must contrast the attempts by Delmas et al (1999) to characterize statistical literacy as statistical, graphical or technical competency with researchers and educators (Gal, 2002, Schield, 2005; Watson, 2006) who characterize statistical literacy as a much wider analytical and critical literacy. In this definition statistical literacy focuses on understanding what is being presented, asking good questions and then evaluating arguments, As Schield asserts; “statistical literacy is more about questions than answers” (Schield, 2005)

2. Material and Methods

The methodology used in this study involved a combination of descriptive and quantitative, especially operational research (OR). Operational research is the discipline of applying advanced analytical methods to help making better decisions. By using techniques such as mathematical modeling to analyze complex situations, operational researches give executives power to make more effective

decisions and build more productive systems base on data mining and modeling. To gather pure and first hand data, thesis and dissertations were assessed by content analysis. Actually, extracted information from them was compared with standard check list to recognize measure and percentage of accordance of applied statistical methods and sequential statistical analysis approach (SSAA) (Malek Mohammadi, 2009). The level of statistical literacy, reasoning and thinking of graduated students, extracted from questionnaire, were compared with findings of content analysis. These notions were actively supported by standard form such as ILS (Vermunt and Vermetten, 2004), SRA (Garfield, 2003) and SATs (Sorto, 2004; Burrren, 2008). Independent variables were the level of statistical literacy, reasoning and thinking. Applied statistical methods according to SSAA were assessed in term of sex, age, educational level, different majors and universities to make sure we have covered all bases.

To obtain model and modeling, relationship between each cited items were analyzed to guarantee used method and sequential statistics, providing that making optimal benefits to affected populations. Regression and path analysis as advanced analysis were used to obtain accurate results.

The population of this study included agricultural extension and education master and PhD graduated student, (N = 750) in selected seven university in Iran, of which 315 student was selected that appraisal for SSAA. Also 115 graduated students were asked by questionnaires to extract another variable. The research based on the Cochran formula and using stratifies random sampling, questionnaires and checklists. Questionnaires face validity was established by a panel of experts consisting of faculty members and graduate students at Tehran University and Islamic Azad University, Iran. A pilot test was conducted with 25 students in the same field. Questionnaire reliability was estimated by calculating Alfa Cronbach, Ordinal Theta and Compose Reliability methods by spss, R and Lisrel software. Reliability for the overall instrument was estimated at 0.91, 0.93 and 0.90 % respectively. Also, questions that decrease each of above coefficients eliminate.

3. Results

Table 1 shows the Summaries of demographic profile and descriptive statistics. The results of descriptive statistics indicated that most of students were men (51.3%). It was reported that slightly more than 83% of Graduated students had master degree whose maximum level of literacy was PhD. Over 84% of them were studied in agricultural extension and education major. Mean their dissertations and thesis marks were 18.93.

Table 1.Personal characteristics of respondent

Variables	Scale	Measure
Sex	Men	51.3%
Degree	Master	83%
Major	Agricultural extension and education	84%
Thesis score	Mean	18.93

Information regarding the factors of statistical principal component of statistics and methodology learning is recorded in Table 2. As can be seen from this, the lowest coefficient variation refers to the level of teacher perceptions about student statistical problems (CV = 2.891) and the highest coefficient variation refers to use of computer in statistical analysis (CV = 7.829).

Table 2. Ranking of principal component of statistics and methodology learning

Options	Mean	SD	CV	Rank
I am not interested in quantitative methods	4.48	1.393	3.216	4
There is not enough real world application in courses	5.03	1.120	4.491	14
I am not good at mathematics and that is why I am not good at methodology	4.78	1.058	4.517	15
Computers are difficult to use when doing analyses	**5.70**	**0.728**	**7.829**	**17**
The teaching is too superficial	5.23	0.956	5.470	16
The teaching is too hasty: there is no time in the lecture to really get familiar with the subjects	4.19	1.176	3.562	8
Examples used in courses are not interesting	3.98	1.304	3.052	3
Methodology skills are easy to forget, because you do not need them daily	4.03	1.242	3.244	5
The data used in courses are not interesting, because they don't feel real own	4.38	1.105	3.963	12
It is hard to see links between different parts of research methodology	4.30	1.092	3.937	11
Methodological concepts are hard to understand	4.69	1.127	3.161	13
Too many new concepts are introduced too fast during courses	4.53	1.202	3.768	9
Teachers use too difficult language and do not explain things well	4.46	1.333	3.345	6
Teachers do not see and understand students problems	**4.14**	**1.432**	**2.891**	**1**
I have a negative attitude toward methodology studies	4.23	1.447	2.923	2
Methodological books are hard to understand	4.90	1.280	3.828	10
Methodology courses need more work that other courses	4.44	1.306	3.399	7

(0 = nothing; 7 = strongly agree)

In order to finding the statistical analysis content knowledge, respondents were asked to express their views. The result showed that the lowest coefficient variation refers to extension, prediction and explanation of extracted information (CV= 3.767) and highest coefficient variation refers to finding of mean, mod and median (CV= 8.429).

The perception of respondents about the statistical reasoning was displayed in Table 3. The lowest coefficient variation refers to Groups can only be compared if they have the same size (CV = 3.079) and the highest coefficient variation refers to Outcome orientation (CV = 5.681).

Table 3: ranking of student statistical reasoning

options	Mean	SD	CV	Rank
Correct reasoning scale				
Correctly interprets probabilities	4.59	1.13	4.02	11
Understands how to select an appropriate average	4.87	0.86	5.53	15
Correctly computes probability, both understanding probabilities as ration, and using combinatorial reasoning	4.68	1.16	4.01	10
Understands independence	4.76	1.24	3.81	8
Understands sampling variability	5.16	1.10	4.66	13
Distinguishes between correlation and causation	4.94	1.05	4.67	14
Correctly interprets two-way table	4.54	1.21	3.74	7
Understands the importance of large sample	4.85	1.31	3.69	5
Misconception scales				
Misconceptions involving averages	5.11	1.25	4.07	12
Outcome orientation	**5.13**	**0.90**	**5.68**	**16**
Good sample have to represents a high percentage of the population	4.85	1.25	3.85	9
Law of small numbers	5.04	1.57	3.42	3
Representativeness misconception	4.67	1.24	3.74	6
Equiprobability bias	4.92	1.40	3.49	4
Groups can only be compared if they have the same size	**4.01**	**1.30**	**3.07**	**1**

(0 = nothing; 7 = strongly agree)

The perception of respondents about the statistical thinking was displayed in Table 4. The lowest coefficient variation refers to" Evaluate published reports that are based on data by examining the design of the study, the appropriateness of the data analysis, and the validity of conclusions "(CV= 3.804) and the highest coefficient variation refers to measurement of univariate (CV= 6.115).

Table 4: ranking of student statistical thinking

Options	Mean	SD	CV	Rank
Understand the differences among various kinds of studies and which type of inferences can legitimately be drawn from each	4.83	0.99	4.83	9
Know the characteristics of well-designed studies, including the role of randomization in surveys and experiments	4.92	0.99	4.95	10
Understand the meaning of measurement data categorical data, of univariate and bivariate data, and of the term variable	5.06	0.89	5.67	12
Understanding histograms, box plots, and scatter plots and use them to display data	4.93	0.96	5.12	11
Compute basic statistics and understand the distinction between a statistic and a parameter	4.80	1.12	4.26	5
For univariate measurement data, be able to display the distribution , describe its shape, and select and calculate summary statistics	**5.95**	**0.97**	**6.11**	**13**
For bivariate measurement data, be able to display a scatter plot, describe its shape and determine regression coefficients, regression equations, and select and calculate summary statistics	4.90	1.09	4.47	8
Recognize how linear transformation of univariate data affect shape center, and spread	4.95	1.13	4.34	6
Identify trends in bivariate data find functions that model the data or transform the data so that they can be modeled	4.92	1.20	4.10	4
Use simulations to explore the variability of sample statistics from a known population and to construct sampling distributions	5.04	1.31	3.83	2
Understand how sample statistics reflect the values of population parameters and use sampling distributions as the basis for informal inference	4.84	1.19	4.04	3
Evaluate published reports that are based on data by examining the design of the study, the appropriateness of the data analysis, and the validity of conclusions	**4.93**	**1.29**	**3.80**	**1**
Understand how basic statistical techniques are used to monitor process characteristics in the workplace	5.10	1.16	4.38	7

(0 = nothing; 7 = strongly agree)

Also, the perception of respondents about the statistical perception and attitude showed that the lowest coefficient variation refers to negative sense about statistics (C= 1.863) and the highest coefficient variation refers to benefit and suit of statistics in professional life (CV = 6.696).

Correlation between variables:

Spearman coefficient was employed for measurement of relationships between the applying proper statistical methods and statistical literacy, reasoning, thinking, perception and several phases of SSAA and graduated student demographic characteristics. Table 5 shows the results which show that there were relationships between apply proper statistical methods and level of study, statistical literacy, reasoning, thinking and attitude. Spearman coefficient was also employed for measurement of relationships between the statistical literacy, reasoning, thinking and different phases of SSAA. Table 5 shows that there were significant relationship between independent variables and dependent variable except for three statements.

Table 5: Correlation measures between independent variables and Applying statistical methods

First variable	Second variable	r	Sig
Statistical literacy (first level) (DD1)	APSM (B)	0.391**	0.00
Statistical literacy (second level) (DD2)	APSM (B)	0.495**	0.00
Statistical literacy (tertiary level) (DD3)	APSM (B)	0.474**	0.00
Statistical literacy (fourth level) (DD4)	APSM (B)	0.394**	0.00
statistical reasoning (C)	APSM (B)	0.617**	0.00
Statistical thinking (F)	APSM (B)	0.273**	0.003
Statistical attitude (E)	APSM (B)	0.591**	0.00
Principal component of learning (A)	APSM (B)	0.394**	0.00
First phase of SSAA (X14)	APSM (B)	-0.229*	0.014
Second phase of SSAA (X15)	APSM (B)	-0.128*	0.049
Tertiary phase of SSAA (X16)	APSM (B)	-0.326**	0.000

*: $p<0.05$; **: $p<0.01$

(B) Applying statistical methods as dependent variable, (C) Statistical reasoning, (DD1) Statistical literacy (level 1), (DD3) Statistical literacy (level 3), (E) Statistical attitude , (F) Statistical thinking , (X14) Initial phases of SSAA, (X15) Intermediate phases of SSAA, (X16) Advanced phases of SSAA

Regression analysis:

Table 6 shows the result for regression analysis by stepwise method. Independent variables that were significantly related to the applying proper statistical methods were subjected to regression analysis. The result indicates that 66% of the variance in the applying proper statistical methods could be explained by two variables of statistical reasoning and statistical attitude.

Table 6: Multivariate Regression Analysis (applying proper statistical methods as dependent variable)

	B	Beta	T	Sig
Constant	-0.24		-0.09	0.92
Statistical reasoning	0.41	0.52	7.93	0.00
Statistical attitude	0.43	0.35	5.04	0.00

$R^2 = 0.66$

Path analysis:

By using Lisrel 8.5 software, path analysis has been done to know the direct and indirect effect of all significant independent variables on dependent variable. Result extracted from path analysis shows that statistical attitude had maximum total effect (direct and indirect effect) on applying statistical methods as dependent variable (Table 7). This mean the weight of statistical attitude to determine dependent variable variance is 0/80, also model fit range from acceptable to weak(RMSEA) (X2/df ratio and p- value) to good (CFI, GFI, AGFI and NFI (Table 8 and Fig. 4).

Table 7- direct, indirect and total effect in path analysis

Paths	Direct	Indirect	Total
From statistical reasoning to applying statistical methods C → B	0.36	-	0.36
From statistical attitude to applying statistical methods E → B	0.66	0.15	0.81
From statistical literacy (level 1) to applying statistical methods DD1 → B	0.49	-	0.49
From statistical literacy (level **3**) to applying statistical methods DD**3** → B	0.80	-	0.80
From statistical thinking to applying statistical methods F → B	-	0.27	0.27
From first phase of SSAA to applying statistical methods X14 → B		-0.32	-0.32
From second phase of SSAA to applying statistical methods X15 → B	-0.34	-0.08	-.42
From third phase of SSAA to applying statistical methods X16 → B	0.41	-	041

Table 8: Suitability indicators in path analysis applying proper statistical methods

Goodness of fit test	Amount
Normal theory weighted least squares chi-square	38.92
P-value	0.020
Degrees of freedom	23
Root Mean Square Error of Approximation (RMSEA)	0.080
Comparative Fit Index (CFI)	0.98
Normal Fit Index (NFI)	0.95
Goodness of Fit Index (GFI)	0.94
Adjusted Goodness of Fit Index (AGFI)	0.85

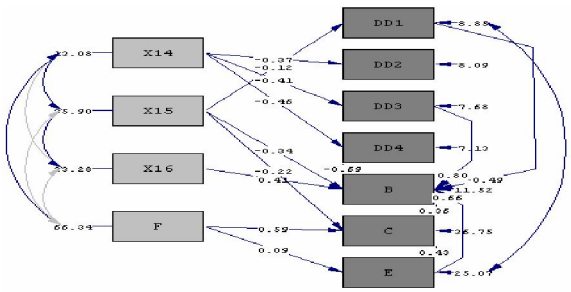

Figure 4: path diagram of direct and indirect effects of independent variable on applying statistical methods

4. Discussions

This paper is intended to be a concise guide for choosing a statistical test with regard to notions extracted from SSAA and statistical literacy, reasoning and thinking. It can use for educational assessment, interpreting and analyzing educational studies without relying on mathematical theories. To provide a framework for understanding statistical concepts and to illustrate the decision-making process to choose a statistical test, we've presented an educational intervention detailing the hypothesis testing, data analysis, and interpreting the results. All notions have shown as a model like figure 4. In this model, each phases of SSAA become meaningful through the components of statistical literacy. Initial phase is recognized as statistical literacy that can be matched with hidden concepts on it. Intermediate phase can be matched with literacy reasoning (Figure 4).

The findings have shown that the students had used statistical phases in regard to their domination on each level of statistical literacy, reasoning and thinking. Meanwhile, applying this roadmap could improve their statistical knowledge. Considering and using sequential statistics by agricultural extension and education students, could give them a general view to exploit from mixed statistical tests. Student could see statistical test in the system and conduct them to understand superior realize from relationship between level and phases and suppose them as group of interrelated, interacting or interdependent elements that forming a complex whole.

Finding synthetic test enable student to refine data and variables. Ultimately, they could extract pure result and knowledge.

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Study of the nutritional value of Persian Gulf squid (*Sepia Arabica*)

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Abstract: Cephalopodan are a group of mollusks that have substantial geographical distribution .Squid have largest fisheries value between Cephalopoda In the world. In the Persian Gulf and Oman Sea are also squid. Due to good taste and friendly meat market, exports this species has three million dollars Currency returns in year1386. Fish meat there are the unique characteristics, including high protein content, unsaturated fatty acids (EPA, DHA), vitamins and minerals thus Fish consumption in the diet is essential. Marine biologists have extracted the new combination of some aquatic that has significant effects in prevent and treat certain illnesses. Information about the Persian Gulf is very limited in this study the nutritional value of squid was investigated. Results showed that this species, with17 percent protein and 8.9 percent fat, having high nutritional value. To protect these stocks should pay more attention to it.

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Keywords: squid, Persian Gulf, nutritional value, sepia Arabica

Introduction

Cephalopoda are old animals and very successful group of mollusks. These animals there are in the different deep all of the world ocean. Between types of Cephalopoda, Sepiidae have high economic value. In Iran's waters, there are many species of the Sepiidae family. Most Cephalopoda of Oman and the Persian Gulf are the kinds of squid. Dominant species of squid in the Persian Gulf is Sepia pharaonis. Squid are observed from the shore to depths of 140 m. but are seen more than 40 meters in depth (fig 1).

Fig1.Persian gulf squid (*Sepia Arabica*)

In recent years the rate of Cephalopoda fishing in the world has increased. Population Increased, has been increased need for food. Aquatic resources, both usual and unusual, are food valuable resources in the Persian Gulf. Identify resources and sustainable exploitation of them is very important. Protein present in the squid meat is high. Some Cephalopoda species have large digestive glands that are rich source of lipid. Fats in aquatic due to unsaturated fatty acids such as (EPA) and (DHA) are an important role in reducing cholesterol and preventing blocked arteries.

Taste like meat Cephalopoda is desirable and non-edible parts it is low, they are the most important sources of seafood. Squid mantle, containing low levels of fatty acids and rich in vitamin C and also a good source of minerals such as calcium, potassium, zinc, iron, phosphate and copper. Cephalopoda not only fresh but also frozen and dried forms are used. Today, production and consumption of frozen squid in the world is increasing. Consumption of these animals in the South East Asian countries is very high. Japan, South Korea, Thailand, Taiwan and China are active in trade Cephalopoda products. According to the latest data from the Iranian fishery, squid per kg price of 3.4 dollars will be issued.

Squid exports in 1386 consisting of three million dollars in currency into the country. The

highest rate of squid countries Spain, France, China and South East Asia countries will be issued. Considering the economic value of squid such research in the nutritional value of, Persian Gulf squid was evaluated.

Materials and methods

Sampled monthly was performed for one year from March 1386 to January 1387 where bahregan estuary with trawl net. Determine the nutritional value, this method was performed that Part from the mantle was separated, at the plate and Moisture content was measured. Determine moisture content, drying temperature of food and determine its moisture content is an indirect method. Petri dishes were in 130 degree oven for 30 minutes. then was cold by desicatore. Petri dishes were weighed by the scale sensitive and Weighed was continued to reach constant weight. 2 gr amount of sample was in the 130 degrees oven for 90 minutes. Sample moisture content, was calculated according to the following formula:

$$\text{Dry matter percent} = \frac{(B - A) \times 100}{W}$$

Moisture percent = 100 - Dry matter percent

W = sample perception weight
B = weight of dry sample + Petri dish
A = weight of empty Petri dish

Methods to ash determine was based destroy sample organic matter, and then the ash was weighing. Calculated was performed based on the following formula:

$$\text{Dry matter percent} = \frac{(B - A) \times 100}{W}$$

W = ash weight + Petri weight
B = weight of empty Petri dish
A = sample perception weight

Fat was measured using soxselea. 2 g of sample powder was in the paper thimble and transferred to extraction apparatus section of soxselea. Then, using the following formula, fat food was calculated.

Fat percentage = balloon weight with Fat - balloon weight without fat / sample weight × 100

Protein measurement method was macrokaldale. In this method, crude protein was measured. (All *proteins* + other materials with N). Then by the coefficient of protein, protein in food was estimated. Because nitrogen in animal protein is about 16 percent and conversion Nitrogen to protein ratio is 6.25. Crude protein content was calculated by the following formula:

CP = percentage of food nitrogen (total nitrogen) × 6.25 (ratio of protein)

In this way, 300 grams of powdered squid mantle with filter paper were inside the digested balloons. Then were added to the balloons 20 ml concentrated sulfuric acid, and 8 g of mixture catalyst (%0.06 dry potassium sulfate, % 3.5 of copper sulfate and %0.5 selenium dioxide). Then was on the stove electric and Temperature increased by slowly.

After cooling balloon in laboratory temperature, and repeated washing with distilled water sample was transferred to the distiller. Under cooling section of distiller, was placed 50 cc Boric acid and reagent. Then was added to, enough sodium hydroxide solution 50%. Distillation continued and all the ammonia produced has been collected in the balloons.

After collecting 300 ml of solution heat was disconnected. Then the solution was neutralized with 0.1 N, HCL. Considering that each cc HCL is equals 0.0014 g N. nitrogen Percent and with regard to protein ratio, samples protein Percent was determined.

Conclusion

Chemical Analysis of squid mantle (Sepia arabica) is given in the below table.

Table 1.Analysis of squid mantle (Sepia Arabica)

Sampling place	Fat (%)	Protein (%)	Moisture (%)	Ash (%)
Persian gulf	**8.90**	**17.00**	**73.02**	**1.00**

Comparison of this study results and similar studies in the Gulf of Thailand showed there are significant differences in terms of nutritional value between Persian Gulf squid and squid in the Gulf of Thailand. In Thailand gulfs quid, protein and fat value have been reported about 14.91 and 0.47 percent. In addition, compared to other Persian Gulf species are shows significant differences in nutritional value. In fact, protein and fat percentage of this species is more than any other species. These differences may be due to different ecological conditions. In other words, perhaps food availability

and type of food is causing these differences. In Similar ecological conditions, species differences are the main factor in the incidence of biological differences, including nutrition.

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Effect of Some Chemical Compounds on Sedimentation Rate of Different Yeast Strains

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Abstract: Heavy metal pollution represents an increasing problem in industrialized as well as developing countries. Yeast cells are capable to accumulate these pollutant from different environments. In this investigation, eight baker's yeast strains were collected from different Egyptian markets. The source of these yeast strains were Misr Yeast, Alinson, Vahine professional, Fermipan, Hollandia Saf–instant, H.u.G and Pakamaya. These strains were grown on basal medium or in molasses medium to determine their efficiency in the bioaccumulation of some metals. The sedimentation measurement was carried out at different salt solutions and different times intervals. The results clearly indicated that SnCl2 followed in descending order by Pb (CH3CooH)2 and AgNO3 were the most effective compounds in increasing the rate of sedimentation of all the tested yeast strains. In contrast; the lowest Figures were recorded with KH2PO4 ,FeCl3 , NiSO4,Co(NO3)2,CaSO4,MgSO4 , Zn SO4, Al2(So)4 and Co CL 2. Other minerals showed a moderate sedimentation capability. It can be stated that yeast cells have a considerable capability to uptake Zinc and iron from the growing medium whereas, manganese showed moderate capability. The lowest values were observed in2the case of copper and lead. *Saccharomyces cerevisiae* can be used as a bioremediation agent for removing heavy metals from the surrounding environment due to its high uptake capacity, taking in consideration that it must be economically competitive with existing technologies.

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Keywords: Effect; Chemical Compound; Sedimentation Rate; Yeast Strain

1. Introduction:

As early as 1000 BC, the Roman, Phoenicians and other early civilizations recovered copper from waters, which had passed through mining operations and ore bodies. In the 1700s, the Spanish at RioTinto applied leaching process to extract copper from copper-bearing minerals. Microbial metal recovery holds great promise for low cost treatment of metal-contaminated industrial effluents and economic recovery of valuable metals, Brierley *et al.*, (1985); Ross (1990).

Fungi and yeasts can accumulate heavy metals and radionuclides even from dilute external concentrations ,Gadd,(1989). Yeast cells are capable of carrying out biosorption (sorption can includeboth adsorption and absorption) and describes the movement of a component from one phase to be accumulated in another solid phase, Gadd, (1989), with various heavy metals. The biomass deriving from*S. cerevisiae* coming from brewing industries is a by-product that is possible to be used in the purification of water contaminated with these ions,Omar *et al*, (1996) showed that yeast biomass fromone of city's breweries can adsorb uranium efficiently, up to 204 mmol of this metal per gram of drybiomass. A study comparing the biosorption of Sr+2 by a laboratory strain and an industrial one of *S.cervisiae* has shown that the industrial strain was more efficient,Avery and Tobin(1992).

Spectrographic analyses have shown that yeasts contain various trace elements, e.g. silver, cobalt, chromium, copper, molybdenum, nickel, lead, tin, vanadium, and zinc, Suomalainen and Qura, (1971). In *Saccharomyces cerevisiae* and *Neurospora crassa*, cobalt is transported via the general3cation uptake system ,Fuhrmann and Rothstein (1968); Venkateswerlu and Sastry (1970). Uptake consists of an initial binding to the cell surface followed by active transport across the membrane. Two K+ are released for each Co +2 Taken up ,Norris and Kelly(1977). Copper and mercury-tolerant strains of *S. cerevisiae* produced more H2S than do their non tolerant parent strains, the metal being precipitated as insoluble sulphides (Ehrlich, and Fox 1967; Kikuchi, 1965, and Naiki, 1957). Electron micrographs have shown that the copper sulphide was chiefly deposited in and around the cell wall, Ashida (1976) ;Gadd and Griffiths (1978).

Precipitation within or on cell-walls may be particularly evident with radionuclides such as uranium and thorium. In *S. serevisiae*. Uranium was deposited as a layer of needle-like fibres on cell-walls reaching up to 50% of the biomass dry weight. That such a large amount was bound by the cells implied that additional uranium had crystallized on already bound molecules, Strandberg *et al*., (1981).The present study was undertaken to assess the ability different strains of *Saccharomyces cerevisiae* to adsorb different types

of heavy metals and remove them from the polluted area.

2.Materials and Methods:

2.1.Materials:

2.1.1.Yeast strains:

Yeast samples (baker's yeast) were rehydrated twice in malt broth medium (Seifert, 1990)) and left to grow for 2 days. Streak method has been used for isolation. The developing colonies on the malt agar plates (incubated at 30°C for 2 days) were picked up under aseptic conditions, purified. Stock culture slants were maintained at 5°C on malt medium after incubation at 25-30°C for 24-28 hrs. Different baker's yeasts were collected from different market in Cairo, Egypt.They included different baker'syeast produced from different countries being Misr yeast, Alinson, Vahine', Fermipan, Hollandia,H.U.G, Saf-instant and Pakmaya.

2.1.2.Standard inoculum

It was prepared by double activation of yeast cells in Erlenmeyer flasks (250 ml in volume) containing 100 ml of basal medium with 1 ml of yeast broth culture 24 hr old to make sure that yeast cells in log phase. The culture should be contained approximately 5X 107Cells/ml (Becker et al., 1990). Baker's yeast strains Were grown on basal medium consists of: 5g Ammonium Sulphate, 10g Bacto-dextrose, 1g KH_2PO_4, 0.5g $MgSO_4.7H_2O$, 0.5-1g yeast extract /L (pH 3.9)on shaker at 150 rpm for 48 hr at 300C.

2.1.3. Salt solutions:

Five percent salt solutions were prepared from CuSO4.5H2O (extra pure Merck), MnSO4.H2O (typeanalysis, Riedel de Haen)MgSO4.7H2O (extra pure Merck), Na3PO4.12H2O (GR Merck), (NH4)2Cr2O7 (pure Koch Light), CaCl2 (anhydrous, Prolabo France), NiCl2(anhydrous for synthesis Merck), KH2PO4(extra pureMerck), Co(NO3)2.6H2 (GR Merck), FeCl3.6H2O (pure Merck),FeSO4 7H2O(GPR Winlab), Al2(SO4)3. 18H2O (extra pure Merck),(Zn SO4 7H2) (analytical grade Nentech,CsCl (ultra pure optical grade BRL),CdSo4.8H2O (type analysis, Riedel de Haen) ,molybdic acid about 90% MoO3(containing ammonium molybdate)(Prolabo France), Pb(CH3COO)2 .3H2O(type analysis, Riedel deHaen),AgNO3,(ACS, Fisher), NiSO4. 6H2O(May and Baker) and K 3 As O4 (analytical grade, Germany).

2.2.Methods:

1% VO SO4. 5H2O(research grade, serva)-2.5% SnCl2. 2H2O (analytical grade, Prolabo France). The pellets were dissolved in HNO3 (Analar , BDH) heated at 60°C until water evaporated and 5 g of the generated salt dissolved in distilled water up to 100 ml.

Four ml of liquid culture was added to five replicates of 25ml volumetric cylinder with stopper. And one ml of tested salt solution was added using a syringe dispenser, (in the case of SnCl2 .2H2O and VO SO4.5H2O 2ml and 2.5 were added respectively instead of one ml) to each cylinder then shake well and let to sediment.

Sedimentation rate (ml/h) was determined after a time range of 1-4 h from the 5 beginning of experiment to evaluate the capability of different chemical compounds and heavy metal solutions to flocculate yeast cells of different strains.

The selected yeast strains which showed the highest efficiency in the bioaccumulation of metals were grown on molasses medium (Abdel-Hafez, 1981) for 6 hours, at 30 C on 150 rpm shaker and the heavy metal were determined using the atomic absorption

3.Results:

3.1.Sedimentation Rates of yeast cells :

It was found that SnCl2 followed in descending order by Pb(CH3COO)2 and AgNO3 are the most efficient compounds in enhancing yeast cells sedimentation. Where the sedimentation rate were 35, 30 and 15 ml/h respectively after 2-3 hours. These yeast strains were the most active baker's yeast in the sedimentation studies as compared with other yeast strains. On the contrary, other heavy metals did not show any effect on yeast cells. The obtained results clearly indicate that solution (2.5%) of Sn Cl2 followed in descending order by solutions (5%) of Pb(CH3COOH)2 and AgNO3, are the most effective compounds in increasing the rate of sedimentation of all tested yeast strains giving >36, >30 and >15 ml/h after 1 hour respectively (Figs.1a, 1b, 1c and 1d). In contrast, the lowest sedimentation rates were obtained with KH2PO4, FeCl3,NiSO4, Co (NO3)2, CaSO4, MgSO4, ZnSO4, Al2 (SO4)3 and CsCl when they gave rates of sedimentation less than 1.0 ml/h. Other compounds are considered to be of a moderate sedimentation rates around 1.0 ml/h.(Fig.2)

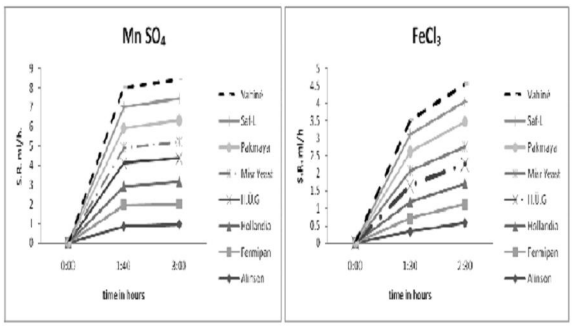

Fig.(1a): Effect of some salt solutions on the sedimentation rate of the different yeast strains

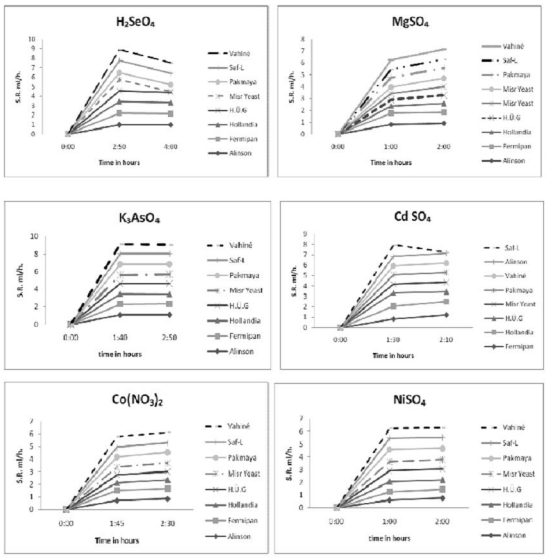

Fig.(1b): Effect of some salt solutions on the sedimentation rate of the different yeast strains.

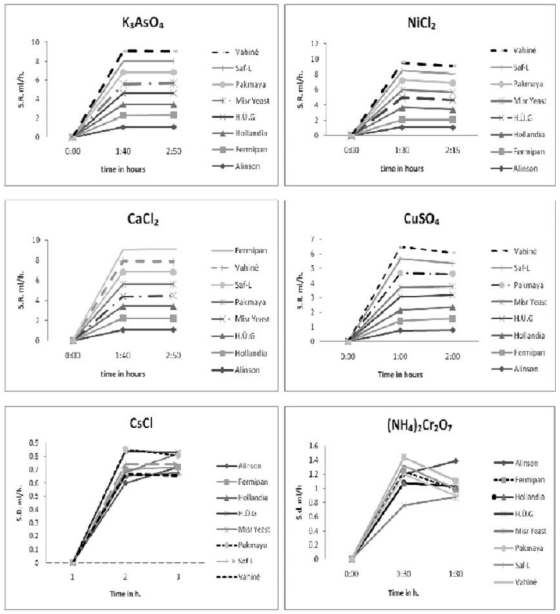

Fig.(1c): Effect of some salt solutions on the sedimentation rate of the different yeast strains.

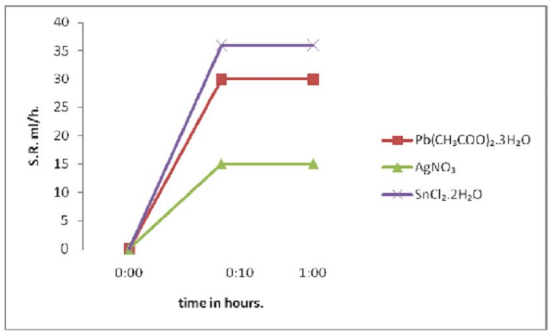

Fig.(2): The most effective salts on the sedimentation rate of all yeast strains.

3.2. Evaluating the capability of *S. cerevisiae strains* (Misr yeast and Fermipan) to uptake heavy metals:

It was found that both tested yeast strains have the ability to accumulate zinc and iron within their cells more than other tested heavy metals namely manganese, copper and lead (Figs.3 and 4). Fermipan yeast strain gave 100% uptake of iron and zinc after 6 h. at 300c, in comparison to only 35.8% uptake of lead. Misr yeast strain gave nearly similar results to those of Fermipan where it showed 100 and 98.6% accumulation of zinc and iron respectively after the same incubation period. Copper proved to be the lowest metal in its affinity to accumulate in the growing yeast cells. In the two yeast strains under investigation, manganese followed iron and zinc descendingly in its affinity to accumulation in both cases.

Fig.(3): The percentage of metal uptake in the yeast cells.

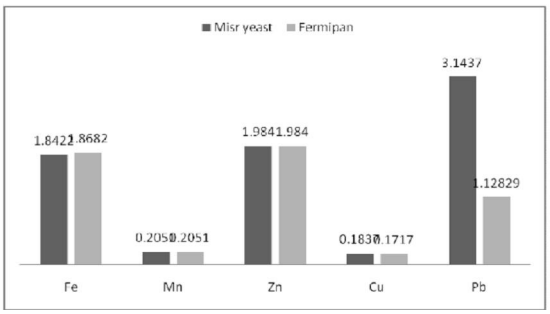

Fig.(4): The uptake of metals in yeast cells in ppm.

4.Discussion:

To elucidate the interaction between 8 yeast srains and 25 different chemical compounds (including some heavy metal ions) the edimentation rate was determined after different periods of time. Kuyucak and Volesky (1988);Mullen *et al*, 1989; McLean and Beveridge(1990) listed the factors affecting metal adsorption by different microorganisms in the following:

a) Composition of biomass.

b) Physical and chemical factors such as the presence of other anions and cations can also affect adsorption either by precipitation (phosphates-hydroxides) or by competition for adsorption sites.

c)Biomass concentration.

d) Living and dead cells.

Regarding the bioaccumulation of heavy metals yeast cells determind by using atomic absorption technique, the obtained results clearly showed that molasses content of zinc followed by iron have a high ability to accumulate within the growing cells of *Saccharomyces cerevisiae*, while others such as manganese has a moderate ability to accumulate in yeast cells. Brierley (1990); Brich & Bachofen (1990); McLean & Beveridge (1990); Volesky (1990), Avery *et al* (1992); McQuanttie *et al* (1992); Denny & Ridge (1995)); Ramirez *et al* (1996); Leyval *et al* (1997); Joner & Leyval (1997) and Childress et al7(1998) demonstrated that different microorganisms can accumulate heavy metals from their external environments.

The slight difference in the present study between the capabilities of different yeast stains to accumulate heavy metals occurred in molasses is previously interpreted by Somers (1963) who noticed that cell wall of fungi and yeast is vary considerably in their overall composition. He added that differences in uptake capacities may exist between different species, between cells of different ages, and even between different cell forms of the same organism e.g., *Penicillium italicum* spore walls take up more copper than vegetative cell walls did.

In the present study, obtained results show that growing yeast strains on molasses medium for only 6 hours gave the maximal metal accumulation. In the same direction, De Rome and Gadd (1987) observed that yeast cells accumulate more metal ions at low cell densities than at high cell densities.

On the light of the our obtained results, it can be generally concluded that *Saccharomyces cerevisiae* can be used as a bioremediation agent for removing heavy metals from the surrounding environment due to its high uptake capacity, taking in consideration that it must be economically competitive with existing technologies.

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Evaluate Area for Very Large Integrated Digital Systems Based on Bandwidth Variation

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Abstract: In this paper, Network on Chip is used as an alternate approach for very large integrated digital systems (System on chip) that is based on bus communications and IP interconnections. This approach has solved some problems like scalability that buses encounter them. One of the basic steps in this approach is correct simulation of NoC implementation; moreover, simulation design operability and perform ability require its synthesizability. Designing and implementation of NoC communication are presented in this work. Finally, bandwidth variation effect on area requirements is evaluated, and area requirements changing due to these alternations will be discussed and explained.

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Keywords: Network on Chip, IP interconnection, bandwidth variation effect, scalability, perform ability.

1. Introduction

Power and performance are two essential features which in corresponded with each other, produce main concerns in design and implementation. Nowadays, very large integrated digital systems [Benini (2005), Chen (2003), Pende (2005), Eisley (2004)] (Systems on Chip) may contain different components such as processor, input- output units and different types of memories. Likewise, each of these components may include different specifications such as variable bandwidth, buses and different communication protocols. Generally, bus is utilized for interconnecting the processing elements of System on Chip (SoC). However by increasing the number of processing elements, the bus itself is transmuted into a bottleneck. To obviate this difficulty, the idea of Network on Chip (NoC) has been introduced [Chiu(2000)].

This network can be modeled as a graph wherein nodes, processing elements and edges are the connection links of the processing elements. In this article, design and implementation of a NoC router are presented. In the second section of this article, the utilized routing algorithm is briefly analyzed. In implementation, XY routing algorithm is utilized [Holsmark (2006), Xiaohu (2007)]. In the third section, the wormhole switching which is used in implementation is reviewed [Duato (1993), Hsh (1992)]. In the forth of this article, the utilized traffic pattern is briefly explained. In the fifth section which considers being the main body of this article, handshaking communication mechanism is introduced and analyzed. In this section, the structure of information packets, router function and different states of the router are analyzed. Furthermore, the experimental results of implementation and synthesis of this routing are presented in the final section of this article. In this implementation, handshaking communication protocol is utilized to interconnect different processing elements.

2. The Utilized Routing Algorithm

The utilized topology for implementation is an $n \times n$ regular two dimensional mesh. A sample of this topology is shown in Figure 1.

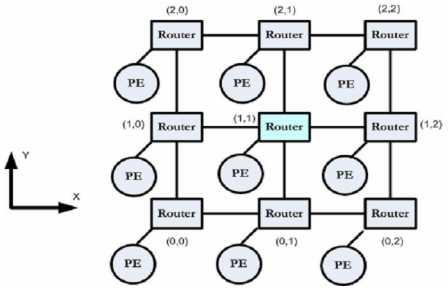

Figure 1. A regular 3×3 mesh topology

The elements which are shown in rectangles represent NoC routers and those which are shown in circles represent the processing elements of this network. By the use of communication links and routers, these processing elements which are connected to each other communication information. Routers are named based on their position in coordinate system. Router ports are also named based on their geographical direction.

However, as it is shown in Figure 1. the number of the ports connected to each other is different due to its position in topology. For example, the router which is placed in the northeast of topology in 2×2

coordinates ([2, 2]), possesses 3 ports and the router in the center of the topology in 1×1 coordinate ([1, 1]), has 5 ports.

For n- dimensional mesh topologies in NoCs, dimension order routing produces deadlock- free routing algorithms. These algorithms are very popular, like XY routing (for 2- D mesh). The routing algorithm which is used in this design is a version of XY algorithm. This algorithm is deterministic algorithm in which packet takes routing in one dimension and it continues till this packet attains the desired coordinate in that dimension. Then routing is fulfilled in the same way. This method warrants no deadlock to occur [Duato (1993), Hsh (1992)]. In this algorithm, according to the coordinates of each router and destination address, routing takes place first in X direction and then in Y destruction and may not be able to adopt a substituting router. It is due to the fact that these types of algorithms adopt routing only based on the source- destination address of packets. Therefore, two packets with the same source and destination address necessarily cross the same route and do not consider the momentary traffic in the route.

3. The Utilized Switching

The need to buffer complete packet within a router can make it difficult to construct low area, compact and fast routers. In implementation, wormhole switching is used which is utilized in almost all of NoCs [Duato (1993)].

In wormhole switching, message packets are also pipelined through the network. A message packet is broken up into flits that the flit is the unit of message flow control. Therefore, input and output buffers at a router are typically large enough to store a few flits [Hsh (1992)].

As we said, in this switching, message packets are divided into equal smaller sections named as flit. Flits are concurrently transferred in the network. Therefore if 16- bit flits are ready to be transferred, 32 signals between two routers are considered to transfer the flits, 16 signals for sending and 16 signals for receiving. In this way, flits are transferred in parallel. Other switching techniques are not commonplace in NoCs usages. For instance, circuit switching technique due to its low performance contradicts with power and performance parameters. Similarly packet switching as a result of its big buffers requirement shows the same contradiction.

4. The Utilized Traffic Pattern

The traffic model is one of the important parameters in evaluating the latency time of interconnection networks. These models are produced according to the application programs which are run on the machine. In different applications, different models are used. Traffic models are defined according to three parameters [Hsh (1992)]: a) The entrance time to networks b) Message length and c) Address distribution type.

The uniform traffic model is the simplest traffic model which used in most of evaluations (and this paper implementation). In this model, each node sends message to the other nodes in network with equal probability. For example in a 6×6 mesh topology, each nodes sends message to the other nodes with the probability of %2.85. All source or destination nodes are selected with equal probability. The selection of source and destination nodes for each message will be independent from other messages [Hsh (1992)].

5. Asynchronous Communication Mechanism

For making interaction between routers, handshaking communication protocol is utilized in case the data is put on the line; the existence of the data is informed to the next router. Next router takes the data from the line and transmits its confirmation to the sender router. So in addition to the flits sending and receiving channels, TX, ACK- TX, RX and ACK-RX signals are required. TX pin is the output and whenever the data is ready in the output port, this pin equals to one and waits for ACK- TX to be equaled to one. Likewise each input port after finding the RX input pin to be one, reads the data on this port and equals the ACK- RX output pin to one.

5.1 The structure of information packets

In each communication standards, the communication payload contains a series of control fields. These fields can be put in the main frame as the redundant fields in order to increase the controllability, fault tolerance, security and some other issues like these. In our intercommunication protocol, flits are used to structuralize. A flit structure is considered in the way that the first bit shows the flit to be the header- trailer or the data. When the first bit equals one, this flit is a header or trailer. In this case, the 2^{nd} bit determines which one is the header and which one is the trailer. This representation is shown in Table 1.

Table 1. The defined protocol that characterize the flit type

First bit	Information type	Second bit	Information type
0	Data	*	Data
1	Header/Trailer	0	Trailer
		1	Header

5.2 Routing function

Each router by receiving the header flit from input, accomplish routing and updates routing Tables according to its source and destination addresses based on XY algorithm.

Henceforth, all of the flits take routing based on the Tables till receiving the final flit (trailer). Routing Tables conclude two Tables: routing Table and output Table. The first Table represents the out port for each input and the second represents the state of each out port (busy or free). In Figure 2. you can see a NoC central router in mesh topology. The central router has 5 I/O port. The local port is utilized to connect the correspondent circle to the processing element (IP block) and other ports are for connecting to other routers.

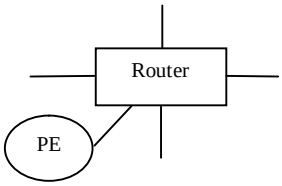

Figure 2. Central NoC router in mesh topology with its ports

The main point here is that the correspondent circle with this routing should have the same interface to be able to use this routing.

Routing function feature takes the charge of routing based on routing algorithm and selection function feature under takes the responsibility of choosing out port in competition circumstances based on the defined priority mechanism. In our designing, mechanisms is implemented by the software in the manner that it gives priority to input port and whatever an input port has a higher priority. It selects its desired output port faster. However, we should consider that competition circumstance only take place when in one moment, there is a request from two input port for one output port.

Our fulfilled designing is implemented by the use of VHDL hardware describing language. In order to router implementation, one entity is designed for whole routing. In code segment of Figure 3. size and type of I/O port are shown.

```
Entity router is
Port(
Clock: in std_lolgic;
Reset: in std_logic;
Data_in: in arrayPortsRegisters;
Rx: in PortsRegisters;
Ack_rx: out PortsRegisters;
Data_out: out arrayPortsRegisters;
Tx: out PortsRegisters;
Ack_tx: in PortsRegisters);
End router;
```

Types of array Ports Registers and Ports Registers signals are defined in one packet. In order to implement, we defined a machine of definite state for input which you can see in Figure 4.

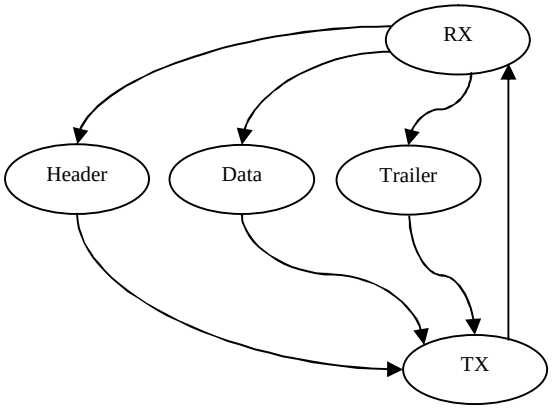

Figure 4. Finite State Machine for flit and router status analyze

5.2.1 Received state

In this state, the routing await for its RX base to be one. In case this happens, firstly the data in Data-in is need and then the correctness of this data is examined. In case of being correct, ACK- RX equal one. Then the next state is defined according to the header/trailer bit.

5.2.2 Header received state

In this state, the appropriate output port is defined based on the source and destination addresses and out port Table. Then routing Table and out port Table are updated. Finally we alter routing state to transmit state.

5.2.3 Trailer received state

In this state, after the destination port is determined by the routing Table, this Table of out port Table is updated. In order to do this, the home correspondent with the input is equaled to NO PORT and also the output port state in out port Table is equaled to free.

5.2.4 Data received state

In this state, after finding the output port by routing Table, the received flit is put in the output port.

5.2.5 Transmit state

In this state, after placing the flit in the output port and equaling the desired output port TX base to one, we wait for receiving ACK- TX and after it's receiving, we equal TX to zero and turn back to the received state.

6. Experimental results

All of the designs which are already presented for NoC, can be used in case they are synthesized. One of the parameters that challenges NoC design synthesizing is the area requirement. For example, many of the presented designs could not be synthesized on the ASIC platform. Table 2 shows the comparison between this article's designed router and other routers. This Table compares some parameters such as topologies, routing algorithms, flit sizes, synthesizability and implementation. As it is obvious from this Table, many of the routers are not synthesized and implemented on ASIC infrastructure. Our router is synthesized and implemented on FPGA as well as ASIC. TSMC 65n is used for ASIC and Spartan 3E is utilized for FPGA.

In order to test the router, a test bench is designed that can send packets from input ports in a uniform traffic pattern and save the output packets in output ports. In the best situation, the Receive state duration, Header- Received, Trailer- Received and Data- Received are one clock cycle. The Transmit state duration is two clock cycles.

Table 3 shows the area requirement for synthesizing the 8 bit designed router on Spartan 3E.

Utilizing percentage of Spartan 3E resources by the 8 bit router is shown in Table 4.

Table 5 shows the area requirement for synthesizing the 16 bit designed router on Spartan 3E.

Utilizing percentage of Spartan 3E resources by the 16 bit router is shown in Table 6.

Table 2. Comparison between article's designed router and other router

NoC Routers	Topology/ Routing	Flit Sizes	Implementation and synthesis
Marescaux (2003)	2D torus (scalable)/ XY blocking, hopbased, deterministic	16 bits data + 3 bits control	FPGA VirtexII /virtexII Pro
Xpipes (Dall'Osso (2003))	Arbitrary (designtime)/ Source static (street sign)	32.64 or 128 bits	No
AEthereal- Rijpkema (2003)	2D mesh/ Source	32 bits	ASIC layout
Eclipse (Tortosa (2002))	2D sparse Hierarchical mesh/ NA	68 bit	No
Proteo (Saastamoinen (2002))	Bi-directional ring/ NA	Variable control and data sizes	ASIC layout CMOS 0.18um
SOCIN (Zeferino (2003))	2D mesh (scalable)/ XY source	n bits data + 4 bits control	No
Hermes (Pande (2003))	2D mesh (scalable)/ XY	8 bits data + 2 bits control	FPGA VirtexII
T- SoC (Grecu (2004))	Fat- tree/ Adaptive	38 bits maximum	
QNOC (Bolotin (2004))	2D mesh regular or irregular/ XY	16 bits data + 10 bits control	No
Our Design	**2D Mesh Regular**	**Variable Data And Control bits**	**ASIC (ASL05 and TSM13u) + FPGA (SPARTAN and Virtex)**

Table 3. Total required area for synthesis of 8 bit router on Spartan 3E

Cell	Library	References	Total Area
BUFGP	xis3e	1×1	1 BUFGP
FDCE	xis3e	1×30	30 Dffs or Latches
FDE	xis3e	1×141	141 Dffs or Latches
FDPE	xis3e	1×5	5 Dffs or Latches
IBUFG	xis3e	1×51	51 IBUFG
LUT2	xis3e	1×68	68 Function Generators

Table 4. Utilization percentage of SPAETAN 3E by 8 bit router

Resource	Used	Avail	Utilization
IOs	101	194	52.06%
Global Buffers	1	24	4.17%
Function Generators	548	21712	2.52%
CLB Slices	274	8672	3.16%
Dffs or Latches	176	22100	0.80%

Block RAMs	0	28	0.00%
Block Multipliers	0	28	0.00%
Block Multiplier Dffs	0	2016	0.00%

Table 5. Total required area for synthesis of 16 bit router on **SPARTAN 3E**

Cell	Library	References	Total Area
BUFGP	xis3e	1×1	1 BUFGP
FDCE	xis3e	1×30	30 Dffs or Latches
FDE	xis3e	1×221	221 Dffs or Latches
FDPE	xis3e	1×5	5 Dffs or Latches
IBUF	xis3e	1×91	91 BUF
LUT2	xis3e	1×132	132 Function Generators
LUT3	xis3e	1×174	174 Function Generators
LUT4	xis3e	1×518	518 Function Generators
MUXF5	xis3e	1×2	2 MUXF 5
OBUF	xis3e	1×90	90 OBUF

Table 6. Utilization percentage of SPAETAN 3E by 16 bit router

Resource	Used	Avail	Utilization
IOs	181	194	93.30%
Global Buffers	1	24	4.17%
Function Generators	824	21712	3.80%
CLB Slices	412	8672	4.75%
Dffs or Latches	256	22100	1.16%
Block RAMs	0	28	0.00%
Block Multipliers	0	28	0.00%
Block Multiplier Dffs	0	2016	0.00%

Figure 5. shows the comparison between synthesizing area requirements of the 8 and 16 bit routers on the Spartan 3E.

The designed router synthesizing process is also done on ASIC 65n platform.

Table 7. shows the area requirement for synthesizing the 8 bit designed router on TSMC 65n.

In the same way, Tables 8. and 9. show synthesizing area requirements of the 16 and 32 bit routers on TSMC 65n.

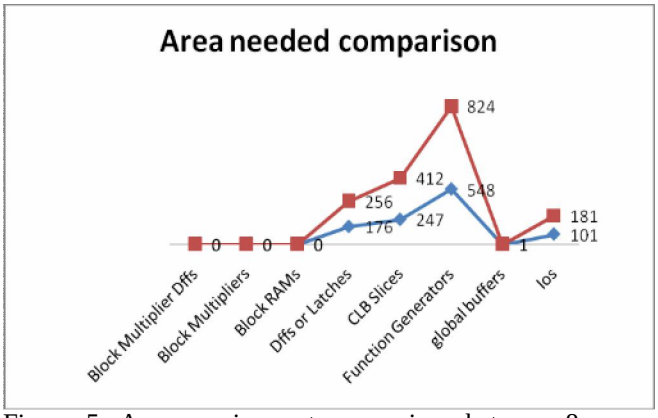

Figure 5. Area requirement comparison between 8 and 16 bit routers for synthesis on Spartan 3E

Table 7. Total required area for synthesis of 8 bit router on TSMC 65n.

Element	Library	Number of Element
Number of ports	umc165sp	108
Number of nets	umc165sp	6616
Number of cells	umc165sp	6554
Number of references	umc165sp	57
Combinational area	umc165sp	16953.120183
Non combinational Area	umc165sp	9302.039932
Net Interconnect area	umc165sp	3.157800
Total cell area	umc165sp	26255.160116
Total area	umc165sp	26258.317915

Table 8. Total required area for synthesis of 32 bit router on TSMC

Element	Library	Number of Element
Number of ports	umc165sp	188
Number of nets	umc165sp	8230
Number of cells	umc165sp	8128
Number of references	umc165sp	60
Combinational area	umc165sp	20388.600205
Non combinational Area	umc165sp	14990.039848
Net Interconnect area	umc165sp	4.176200

Total cell area	umc165sp	35378.640053
Total area	umc165sp	35382.816253

Table 9. Total required area for synthesis of 16 bit router on TSMC 65n.

Element	Library	Number of Element
Number of ports	umc165sp	348
Number of nets	umc165sp	11693
Number of cells	umc165sp	11511
Number of references	umc165sp	64
Combinational area	umc165sp	29047.680285
Non combinational Area	umc165sp	26366.039680
Net Interconnect area	umc165sp	6.276800
Total cell area	umc165sp	55413.719966
Total area	umc165sp	55419.996766

Figure 6. shows the comparison between synthesizing area requirements of the 8, 16 and 32 bit routers on the TSMC 65n.

Based on the presented statistics data, the following results are provided:

1. The effect of bandwidth variation on the area requirements is not linear.
2. The increase rate of area requirement proportion enhances by the bandwidth increase. As it was shown in this article, the area requirement increase proportion of 8 bit bandwidth to 16 bit was 1.34. However, this rate was 1.49 for 16 to 32 bandwidth increase.

Power consumption of implemented router has been analyzed. Results are shown in following Tables (Table 10, 11 and 12). These results belong to 8, 16 and 32 bit routers.

6. Conclusion

In this article not only we used an asynchronous communication mechanism based on handshaking to transfer information but also by using statistical data, we showed that this designed router occupies very little space.

Scalable design of this router leads to easy and efficient addition of new capabilities like 16-bit and 32-bit bandwidth. The resource utilization of this router is more efficient than similar implementation on FPGA and ASIC platforms.

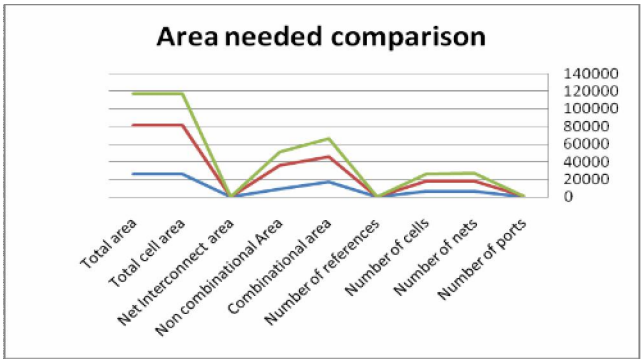

Figure 6. Total accumulated area requirement comparison among 8, 16 and 32 bit routers for synthesis on TSMC 65n (ASIC)

Table 10. Total power consumption information for 8 bit router

	Switching Power(mW)	Internal Power(mW)	Leakage Power(pW)	Total Power(mW)
Router Power	0.157	0.979	5.63e + 08	1.698

Table 11. Total power consumption information for 16 bit router

	Switching Power(mW)	Internal Power(mW)	Leakage Power(pW)	Total Power(mW)
Router Power	0.154	1.484	7.32e + 08	2.370

Table 12. Total power consumption information for 32 bit router

	Switching Power(mW)	Internal Power(mW)	Leakage Power(pW)	Total Power(mW)
Router Power	0.201	2.677	1.15e + 09	4.027

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Changing of Self-Care Behavior by Practicing 12-Step Program among Codependents in Iran

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Abstract: Promoting positive sense of self and taking care of self among people are important factors in order to achieve health promotion in every community. As self-forgetting is special character among codependents, so this study aims to find differences of self-care behavior by comparing families of addicts/alcoholics who practice the "12-step program" and who do not. In other words, this study investigates whether "12-step program" can empower families of addicts/alcoholic to change their self-care style or not. Theory of empowerment is the key theory to conduct this study. The findings of this study indicate that "12-step program" is effectiveness program to enable codependents to having positive self-image. In other words, independent samples t-test reveals that codependents who practice the "12-step program" take care of themselves more than another group who did not practice this program.

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Keywords: 12-Step program; Addiction; Al-Anon & Nar-Anon; Codependency; Families of Addict; Self-care

1. Introduction

Addiction/alcoholism is a major social problem in several countries. In Iran, article 9th of the general policies of drug abuse reduction and 97th provision of fourth economical, social and cultural development program have emphasized on the locals participation in drug abuse reduction and prevention programs. The results of various surveys carried out by the *Islamic Culture Ministry* (2007), showed that addiction is the first social harm in Iran (Iranian Attitude toward Drug Abuse, 2004 & 2008). Although the subject of substance abuse or alcoholism amongst adult people was researched world-wide, there is not much that can be said about codependency syndrome especially in Asia.

Co-dependency may be apparent in various situations. However, it is often most easily recognized in families with chemical dependency (Beattie, 1989). Unfortunately some of the codependents are living in dangerous situations. Their children and themselves may be experiencing violence or living in danger for as long as they live with an addict. The family of an addict gradually becomes trained to anticipate crises events which results in addiction. Consequently, the family members become limited to special conditions for preventing, controlling or minimizing the crises. Controlling the drug abuser or providing for the needs of the addict causes the family members to sacrifice their self-worth, personal growth and needs (Motivational Assessment Process for Families Members, 2002).

Because of living with an addict/alcoholic person, codependents tell themselves that their personal needs and desire are unimportant as compared to the addict's. This kind of thinking gradually becomes a main part of their behavior when dealing with others. As a result, external focus, boundary problems and controlling behavior appear in their relationships with others(Parker & Guest, 1999).

As substance/alcohol abuse creates many difficulties for both the addict/alcoholic and the codependent (Brown & Lewis, 1998), then family members become an important part of recovery programs (Garrett, et al., 1998). They can empower themselves by learning new patterns of thinking, feeling behaving and relating to others. Based on empowerment theory if codependents try to employ useful recovery tools in order to pay attention to their self needs, they will be empowered in their life (Rowlands ,1997) The survey of literature revealed that when it comes to recovery, there are several methods and programs for families of addict/alcoholic. Sequential Families Addictions Counseling Model, Cognitive Behavior Therapy and 12-step program are some of them which help families of addicts/alcoholics.

Among several programs which help those families who lived with an addict/alcoholic, this study has been focused on a program which is called "twelve-step" or "12-Step". The concept of this program is back to the steps designed by Alcoholics Anonymous (AA) which was founded in 1935 (Lowinson, et al., 2004).

Two groups which employ the 12-step

program for members (in the world) are Al-Anon & Nar-Anon. These families groups are founded in 1951 by the wives of two Alcoholics Anonymous members. These are community resources that provide support to anyone affected by a relative or friend's drinking or drug abuse (Fisher & Harrison, 1999).

The fellowship of the group should become the most important tool in the reader's recovery process not only because such groups give the person an opportunity to be with people who are talking about the disease and recovering from it, it is also because a 12-step group is the most likely place to surround him/herself with those who will support him/her in his/her efforts, and those who are striving to live by new rules. Such environments will support their efforts to recover (Greenberg, 1994). Rowlands believed that sharing experience with others who have same problem is one important way to empower a person (ÖSTE, 2003).

As a result, the aim of this research is to examine the result of practicing 12-step program in self-care variable by comparing Experimental group and Control group. In this stage, it is attempted to answer this question: whether there are any significant differences in self-care behaviors between two groups of codependents or not.

2. Methodology

This study was conducted in Shiraz city which is located in the southwest of Iran. As causal comparative design was considered in this research so the design of this study is based on the two sets of data (Gay, Mills and Airasian, 2006).

The first population was located in four branches of Al-Anon/Nar-Anon (meetings) in Shiraz city. The second population is families who did not practice 12-step program. Researcher found these families among families who conducted their addicts/alcoholics to the recovery camps (Javanan camp). Based on research methodology references, in causal comparative research 30 people for each group is an accepted number as sample size (Wallen & Fraenkel, 2001). Consequently, 60 families were selected for this study.

It is considered that, five-point Likert scale ranging from (1) "strongly agree" to (5) "strongly disagree" was employed to measure each item of questionnaire. As the majority items are negatively worded so the score is started from 1 to 5. On the other side, there are several positively worded items which were displayed with * symbol in questionnaire. For these items the five-point Likert scale has been ranged on the contrary of negatively items, from (5) "strongly agree" to (1) "strongly disagree". In other words, all responses of positively worded items were reversed in calculating means.

In causal comparative research it is very critical to select two groups that are homogonous. In other words, groups should be matched to each other on one or more criteria. By this way these important criteria can be controlled by researcher and be sure that only independent variable affected to group (Wallen & Fraenkel, 2001).

As mentioned in the previous paragraph, Use of Tranquilizers by families, participate in other programs (except of 12-step program) and psychotherapy continually were considered as three control variables which were controlled by researcher.

3. Results and Discussions

In present study self-need is the key subject of self-care scale which was considered between families of addicts who practice the 12-step program and those families who did not. Gehert (1993) stated codependents often have an excessive sense of own importance and responsibility. They assume that nothing will get done if they do not handle it. As a result, they repeatedly devote themselves for unnecessary service, and finally they feel no good about themselves. So taking care of the body, mind and spirit will be ignored.

Self-care variable among families of addicts/alcoholics as the main variable in this study was measured in fourteen items by comparing two groups of families of addicts which were differed by practicing 12-step program. Table 1 presents the mean score, standard deviations, t-value and p-value of each item in both Experimental and Control groups.

Table 1. **Self-Care Items**

Statement	Mean	SD	t-value	p-value
I blame myself for my actions				
Ex-group	3.67	1.21	6.84	0.000
Co-group	1.87	0.77		
I strive hard (more than enough)				
Ex-group	3.37	1.24	4.97	0.000
Co-group	1.90	1.02		
I don't care about going to doctor				
Ex-group	3.77	1.13	4.11	0.000
Co-group	2.47	1.30		
*I always dress decent				
Ex-group	4.10	0.84	2.93	0.000
Co-group	3.33	1.15		
*My body health is important to me				
Ex-group	4	1.01	3.53	0.001
Co-group	3.03	1.09		
Nobody respects me				
Ex-group	4.17	0.78	5.86	0.000
Co-group	2.70	1.11		
I am sacrificed by others				
Ex-group	4.40	0.62	15.37	0.000
Co-group	1.63	0.76		

I have no intention to do anything				
Ex-group	4.37	0.71	7.07	0.000
Co-group	2.54	1.22		
I am obsessed by thinking about past and worry about future				
Ex-group	4.40	0.67	19.92	0.000
Co-group	2.53	1.22		
*I feel happy and healthy gradually				
Ex-group	4.20	0.61	9.91	0.000
Co-group	2.37	0.80		
I always focus all of my attention on my addict recovery				
Ex-group	3.97	0.55	8.33	0.000
Co-group	2.1	1.09		
My well-being and happiness depend on addict recovery				
Ex-group	3.90	0.75	12.01	0.000
Co-group	1.60	0.72		
Stress makes me sick				
Ex-group	3.80	0.96	8.93	0.000
Co-group	1.80	0.76		
I can't sleep well				
Ex-group	3.97	0.71	3.87	0.000
Co-group	3.07	1.04		

*. Positively worded item

Independent samples t-test reveals significant differences in mean scores for all fourteen items between families in Co-group and families in Ex-group ($p < 0.05$). These findings show that families who have practiced 12-step program take care of themselves more than another group. On the other side, families who do not practice 12-step program, cannot consider their health or body unlike another group. In experimental group after practicing 12-step program, families learn to look at themselves rather than other people.

The result of Table 1 in present study confirms that families of addict only focus on the addict person. Moreover their well-being and happiness depend on addict recovery. Therefore their central thinking is around addict, so self-care will be dropped. On the other side, families who practice 12-step programs learn to look at within and take care of themselves. In other words, there is significant difference in families' attention toward addict in Ex-group in comparison with families in Co-group.

Bibee (2005) described this issue as the self-victim. He believes self-victim in codependency is special role that is played to keep the game alive. They see themselves helplessness, hopelessness and powerlessness. As a result, they feel whine, blame, and guilt about how hard life is and ask for a rescuer. This study confirms the same results about the mentioned feeling. Families in control group blame themselves because of their addicts. Also these families have a rejection sense by others. They feel other people do not respect them while they have been sacrificed for others' problem. On the other side, families who practice 12-step program not only do not have any blame as a behavior toward themselves, but also they feel that others respect to them. Moreover there is no forgotten feeling by other people among them. Generally this group feels good about themselves rather than another group.

In another study, Harkness, Manhire, Blanchard, & Darling (2007) explored a model of codependent attitude and behavior as moderators of the relationship between (alcohol and other drug) AOD problems in the families of origin (AODF) and offspring self-report of psychological distress. They concluded that codependency may protect adult offspring from "feelings of personal inadequacy and inferiority, particularly in comparison with others."

Based on mentioned points and the result of this study, basically families of addicts/alcoholics conserve their energies for the others' needs and their most important things such as their feeling, health, cloths, body, and sleep will be ignored by forgetting themselves as an independent person. In this situation the 12-step program is an ideal program for codependents. When families of addict as codependents try to recover, they will learn to pay attention to their needs and look at themselves. Based on obtained result in Table 1, self-care among families after practicing 12-step program is significantly more than families who do not practice this program.

4. Conclusion

Health promotion is about helping people to have more control over their lives, and consequently improve their health. It happens during processes of helping and enabling people to strengthen personal skills, constructing supportive situations and developing communities. Moreover this is an approach that represents community development and promotes a positive sense of self. One important part of health promotion approach which needs to consider in every community is addiction/alcoholism. Addiction/alcoholism is one major social problem in several countries. This harmful illness not only effects on addict/alcoholic person but also it effects on the health of his families, relatives and even friends.

So the question is: What activities are likely to improve the overall health status of codependents' population? The implication of this approach is that when families of addict/alcoholic can identify their own health problems, have accessed to the information needed for their solution, and developed the confidence and assertiveness to act, as a result changing may occur at a community, as well as an individual level.

As community development seeks to empower individuals and groups of people, by the skills they need to defend on their own behalf, improve their healthy lives, and consequently self-help groups like "12-step program" can be one of the most important components to reach these goals.

One of the most common themes in the self-help group research is empowerment (Cheung, Mok & Cheung, 2005). This study suggests that empowerment can refer to the occurrence of changes of the individual in personal qualities such as self-care. Al-Anon/Nar-Anon Members gain their personal and social identity by sharing common beliefs, values and norms which have been developed by 12-step program. They develop some knowledge of their identity as a group, and share their problems and usual needs in the group meetings. By this way, they will be able to solve their difficulties by sharing their experience with other members who have same problems in their life. The present study revealed that when Experimental and Control groups were compared to each other, personal empowerment occurred among codependents by practicing 12-step program and it leads to change their self-care behaviors.

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Role of *Atherina* Species in Transmitting some Bacterial Diseases to Human

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Abstract: A total of 530 samples (300 from fresh water marine *Atherina*), 130 samples from water used for preparation of Atherina fish for selling, and 100 hand swabs from their handlers) were collected from randomly selected markets from 3-localities in Sharkia governorate, Egypt. All samples were examined for the presence of *Staphylococcus* species and *Enterobacteriaceae*. Moreover, the effectiveness of freezing, salting, and commercial vinegar (5% acetic acid) treatment on the survivability of *Staphylococcus* spp. and *Enterobacteriaceae* in *Atherina* fish was also evaluated. Results revealed *S. aureus* were detected in 65.7% of the surface swabs and 35.7% of muscle samples of fresh water *Atherina* fish. The prevalence of *S. aureus* in the surface swabs and muscle samples of marine *Atherina* fish were 62.2% and 25.6%, respectively. *Enterobacteriaceae* isolated from surface swabs of *Atherina* fish were; *E. coli* (5.33%), *Kl. oxytoca* (7%), *Kl. pneumoniae* (5.7%) *Ent. cloacae* (5%), *P. vulgaris* (9%), *P. mirabilis* (6.3%), *Sh. sonnei* (1.7%), *Cit. freundii* (5%), *Cit. koseri* (6%), *Pantoea agglomerans* (38.3%), *Hafnia alvei* (1.7%), *M. morganii* (2.3%), and unidentified spp. (8.7%). The percentages of isolation of the previous species from muscle samples of *Atherina* fish were 0.7, 2.3, 1.3, 1.7, 5, 3.7, 0.7, 1.7, 2.7, 24.7, 1.3, 1.3, and 3.7, respectively. The prevalence of *S. aureus* was 53.1% in water samples used for preparation of fish for selling. *Enterobacteriaceae* isolated from water samples were *E. coli* (6.15%), *P. mirabilis* (7.7%), *P. vulgaris* (11.5%), *Ent. cloacae* (7.7%), *Cit. freundii* (6.15%), *Cit. koseri* (6.9%), *Kl. pneumoniae* (7.7%), *Kl. oxytoca* (9.2%), *Pantoea agglomerans* (30.7%), *Hafnia alvei* (2.3%), *M. morganii* (3.1%), *Sh. sonnei* (1.5%), and unidentified species (3.8%). *S. aureus* was isolated from 73 hand swabs. *Enterobacteriaceae* isolated from hand swabs were *E. coli* (5%), *P. vulgaris* (8%), *P. mirabilis* (5%), *Kl. pneumoniae* (6%), *Kl. oxytoca* (7%), *Ent. cloacae* (6%), *Cit. freundii* (4%), *Cit. koseri* (5%), *Pantoea agglomerans* (36%) and unidentified species (18%). Ten representative biochemically identified *E. coli* isolates (4 from *Atherina* fish, 3 from water used for preparation of fish for selling, and 3 from hand swabs of fish handlers) were identified as O_{128} (2-strains), O_{114} (strain), and O_{136} (strain) from *Atherina* fish, O_{26} (strain), O_{111} (strain) and untyped strain from hand swabs of fish handlers. However, all isolates from water samples were O_{128}. The survivability experiment revealed that all muscle samples were negative for all bacteria species growth from the 1st week of freezing. After 1st week from freezing, all *Enterobacteriaceae* were continued to isolate (1:4 each) from the surface swabs of the 4 examined samples. On the other hand, *S. aureus* was continued to isolate at a rate of 4:4 . All *Enterobacteriaceae* except *P. mirabilis* (each with 1:4), *S. aureus* (4:4), Coagulase negative *Staphylococcus* spp. (1:4) were continued to isolate after the 2nd week from freezing. The isolated species after the 3rd week of freezing were *Kl. oxytoca*, *Pantoea agglomerans*, and un-identified species (1:4 each), and *S. aureus* (4:4). *Pantoea agglomerans*, un-identified species and *S. aureus* were continued to isolate after 4th week. The un-identified species (1:4) and *S. aureus* (4:4) were continued to isolate until the week 13 from freezing. *Kl. oxytoca*, *P. vulgaris*, *P. mirabilis* (1:4, each) were isolated from surface swabs of fresh water *Atherina* fish salted in NaCl solution (25%). Moreover, *Pantoea agglomerans* and *S. aureus* were isolated with ratios of (2:4) and (4:4) of the same samples, respectively. On the other hand, the bacterial spp isolated from the muscle samples of fish salted at NaCl 25% were *Kl. oxytoca*, *Pantoea agglomerans* (1:4, each). All samples salted in 50% and 75% NaCl solution were negative for the presence of *Enterobacteriaceae* from the 1st week and for the whole period of the experiment. *E. coli* was continued to isolate until the 6th hours of treatment but stop to grow after 7 hours from vinegar treatment. *S. aureus* was negative in all treated samples from the 1st hour of treatment.

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1. Introduction:

The recent food scares related to ruminant's meat (Bovine spongiform encephalopathy and Scrapie), chicken meat (Avian Influenza), the increased prices of beef and poultry meat, and the dietary changes of the consumers toward healthier food habits have resulted in a rapid rise of seafood consumption worldwide (Yasumoto, 2000).

However, as demands for and production of seafood increased, it becomes important to pay more

attention to seafood safety and seafood related diseases (Gillespie *et al.*, 2001). Center of Disease Control (CDC) and U.S. Department of Agriculture recorded that seafood is 25 times more likely than poultry to cause illness in man (FAO, 2001).

Atherina species are small fishes in coastal areas belonging to family *Atherinidae* which feeds on small crustaceans and fish larvae (Maugé, 1990). They are considered an important source of animal protein especially for poor people in Egypt.

Nowadays, water pollution with domestic waste water is most common in River Nile and its tributaries; the greatest volume of wastes discharged into the water course is sewage. Such pollution reduces the water quality and had been reported as a precursor to fish infection with microorganisms that can infect both consumers and handlers (Omer *et al.*, 2004) either through ingestion of fish meal or through direct contact through abraded skin (Czachor, 1992). Moreover, *Atherina* species are liable for contamination after being harvested either from infected fish handlers or contaminated utensils and equipment during transportation, distribution and food preparation.

Fish may serve as carrier for several zoonotic bacteria as *E. coli*, *Salmonella* sp., *Aeromonas* sp., *Pseudomonas* sp., *Proteus* spp., *Shigella* spp., *Staphylococcus aureus*, *Erysipelothrix* which are incriminated in food poisoning, skin disorders, allergic condition as well as other infection (Janssen, 1970).

Due to the perishable nature of fish, various fish preservation techniques have been developed through history. Both land and marine fish suffer from wide seasonal variation in catch volume. To maintain a stable fish supply during times of scarity and to maintain such fish with low microbial load, drying, salting, fermentation, smoking, addition of antimicrobials and freezing were widely practiced (Al-Jedan *et al.*, 1999). Sodium chloride, trisodium phosphate, lactic acid, and acetic acid have been used as food preservatives are generally recognized as safe (Branen *et al.*, 1990). On the other hand, icing of fish was found to cause a decrease in the aerobic, mesophilic, and psychrotrophic bacteria after 5-days of storage (Lalitha and Surendran, 2006).

2. Materials and methods:

A total of 530 samples (300 from fresh water marine *Atherina*, 130 samples from water used for their preparation for selling, and 100 hand swabs from their handlers) were collected from randomly selected markets from 3-localities in Sharkia governorate, Egypt. All samples were examined for the presence of *Staphylococcus* species and *Enterobacteriaceae*. Moreover, the effectiveness of freezing, salting, and commercial vinegar (5% acetic acid) treatment on the survivability of *Staphylococcus* spp. and *Enterobacteriaceae* in fresh water *Atherina* fish was also evaluated.

I. Occurrence of *Staphylococcus* spp. and *Enterobacteriaceae* in *Atherina* fish, water used for their preparation for selling, and hand swabs from their handlers.

A. Sampling:

1.1.1. *Atherina species*:

Fish samples were identified as marine or fresh water *Atherina* fish according to their characteristic morphology (Maugé, 1990) then placed in polyethylene bags, ice packed and transferred to the laboratory within short time. Surface swabs and muscle samples from each fish were collected according to (Sanaa, 2009) as the followings:

1. Surface swabs:

A sterile swab was rolled over the surface of each fish to be examined and immersed in a tube containing buffered peptone water (BPW).

2. Muscle samples:

The surface of fish was sterilized with a red hot scalpel; a part from the muscle underlying the sterilized surface was then picked up by the mean of sterile spatula and placed into sterile tube containing buffered peptone water.

3. Water used for preparation of *Atherina* fish for selling:

One hundred and thirty water samples that used for preparation of *Atherina* fish for selling were collected from the same localities from which *Atherina* fish were collected. All samples were collected under complete aseptic conditions in sterile colourless glass bottles provided with stopper. The samples were dispatched on ice and transferred to the laboratories with a minimum time of delay. Twenty-five ml of each water sample were added to 225 ml BPW and incubated at 37ºC for 18-24 hours.

4. Hand swabs from fish handlers:

Sterile swabs were moistened in sterile BPW, rolled all over the hand and then immersed into test tubes containing BPW. The tubes were labeled with respect to name, age, sex, and date of collection. The tubes were put on ice, packed and transferred to the laboratory with in a minimum time of delay.

B. Bacteriological examination according to (Cruickshank *et al*., 1975):

1. Isolation and identification of *Staphylococcus* species*:*

It was done according to (Sonnerwirth and Jarett, 1980). Characteristic yellow colonies with yellow zones and small red colonies with no colour change in surrounding medium from each plate of mannitol salt agar (Oxoid, CM_{85}) were purified on nutrient agar (Oxoid, CM_3) slants and incubated at 37°C for 18-24 hours for further identification including:

1.1. Microscopical examination:

Films were prepared from the pure cultures and stained by Gram's stain technique and examined under ordinary microscope (10x and 40 x) to verify the presence of characteristic features of the organisms and to confirm the specificity of the colonies.

1.2. Biochemical identification (Catalase test and Tube coagulase test)

2. Isolation and identification of *Enterobacteriaceae*:

It was done according to Koneman et al., (1997). Characteristic pink colonies and pale colonies on MaCconky agar (Oxoid, CM7) plates were purified on nutrient agar (Oxoid, CM_3) slants and incubated at 37°C for 18-24 hours for further identification including:

2.1. Microscopical examination:

2.2. Detection of motility:

It was detected by stabbing the suspected microorganisms into semi-solid agar tubes containing 0.4% agar and incubated at 37°C for 3 days. Spreading of the growth from the stab line was considered positive.

2.3. Biochemical identification: (Oxidase test, Indole test, Methyl red test, Voges proskauer test, Citrate utilization test, Urea hydrolysis test, Sugar fermentation test, H_2S production, and Decarboxylation of arginine, ornithine and lysine).

2.4. Serological identification

Ten representative isolates of the bacteriologically and biochemically identified colonies of *E. coli* from *Atherinae* fish (No. = 4), water used for their preparation for selling (No. =3), and hand swabs of their handlers (No. = 3) were subjected to serological identification at Animal Health Research Institute, Dokki, Giza, and the Central laboratories of the Ministry of Health and Population, Cairo, Egypt. Serodiagnosis was done by slide agglutination technique (Sojka, 1965) using set 1:O polyantisera and related mono antisera (Denka Seiken Co).

II. Effectiveness of freezing, salting (NaCl), and commercial vinegar (5% acetic acid) treatment on the survivability of *Enterobacteriaceae* and *Staphylococcus* species in *Atherina* fish:-

1. Freezing

Ten *Atherina* fish were randomly taken from a total quantity of one kilogram of fresh water *Atherina* and examined for the presence of both pathogens before freezing process. The remaining *Atherina* fish were divided into 13 parts. Each part was placed into sterile polyethylene bag. Each bag was tightly sealed and kept in deep freezer at -20°C. Four *Atherina* fish from the first bag was thawed and their surface swabs and muscle samples were examined for the presence of *Enterobacteriaceae* and *Staphylococcus* species after one week from freezing. This step was repeated with the remaining bags until 13-week.

2. Salting (NaCl- treatment):

Ten-representative fresh water *Atherina* samples randomly taken from a total quantity of one kilogram of fresh water *Atherina* were examined for the presence of *Enterobacteriaceae* and *Staphylococcus* species before salting treatment. The remaining quantity of *Atherina* fish was divided into 3-groups. The first group was immersed in NaCl solution at a concentration of 25% (wt/vol). The 2nd and 3rd groups were immersed in the same solution with concentrations of 50%, and 75%, respectively. From each groups 4-representative *Atherina* fish samples were picked up, washed with sterile water to remove the NaCl residues then surface swabs and muscle sample of each fish were examined for the presence of *Enterobacteriaceae* and *Staphylococcus* species. This process was repeated at an interval of 1-week for 6 weeks (Ez El-Din, 1999).

3. Commercial vinegar (Acetic acid 5%) treatment:

Ten-representative fresh water *Atherina* samples randomly taken from a total quantity of one kilogram of fresh water *Atherina* were examined for the presence of *Enterobacteriaceae* and *Staphylococcus* species before their treatment with commercial vinegar solution containing 5% acetic acid. The remaining quantity of *Atherina* fish was immersed in vinegar. Four fish samples were picked up and washed with sterile water to remove the vinegar residues. Surface swabs and muscle samples of fish were examined for the survival of *Enterobacteriaceae* and *Staphylococcus* at an interval of 1-hour and for 24 hours (Bin-Jasass, 2008).

3. Results and Discussion:

Atherina spp and other fish species are subjected to many risks of contamination which result from sewage pollution of their aquatic environment or from unhygienic handling during

harvesting, transportation and processing (Ibrahim *et al*., 2009). *S. aureus*, the main cause of food intoxication in man due to consumption of the preformed toxins in various food stuffs, and members of the family *Enterobacteriaceae* are the predominant bacterial pathogens transmitted from *Atherina* spp to man. They are involved in enteritis, urinary and respiratory tract infection as well as pyogenic infection (Novothy *et al*., 2004).

Results in Table (1) clarifiy that the overall prevalence of *S. aureus* infection in surface swabs versus muscle samples of all examined *Atherina* spp. were 64.7% Vs 32.7%.The comparable prevalence of coagulase negative *Staphylococcus* spp in the same samples were 40% Vs 22%. The percentage of *S. aureus* infection in muscle samples of *Atherina* fish is nearly similar to that recorded by Mugula and Lyimon (1992). Lower percentages were previously reported by Wienke *et al*. (1994) in fish and shellfish flesh; Normanno *et al*. (2005) in fish flesh, and Simon and Sanjeev (2007) in flesh of fishery products.

Regarding the occurrence of *Staphylococcus* spp in fresh water *Atherina*, it is clear from table (1) that *S. aureus* was isolated from surface swabs and muscle samples of fresh water *Atherina* with the percentages of 65.7 and 35.7, respectively. The recorded infection rate of *Staphylococcus* spp in the muscle samples of fresh water *Atherina* is in agreement with those reported by Noha and Ghada (2006) in sardine fish and Metawea and Abdel-Ghaffar (2007) in *Tilapia nilotica.* On the other hand, higher infection rate with *S. aureus* (43% and 53%) was recorded by Omaima and El-Kewaley (2008) and Ibrahim *et al.* (2009). In contrary, lower infection rates of 4.35% and 3.93% of *S. aureus* in *Tilapia nilotica* and *Labea niloticus*, respectively were previously recorded by Abo-El-Alla and Bastawrows (1999).

Concerning the occurrence of *Staphylococcus* spp in marine *Atherina* fish, results illustrated in table (1) show that *S. aureus* was isolated with percentages of 62.2 and 25.6, respectively. Mohamed *et al.*, (2001) and Omaima and El-Kewaley (2008) isolated the same pathogen from flesh of shellfish (*Crabs* and *Shrimps*) and *Mugil cephalus* flesh with the percentages of 25 and 32, respectively. On the other hand, Papadopoulou *et al*. (2007) recorded higher infection rate of 80% in the flesh of marine fish.

It was clear from table (1) that the prevalence of *S. aureus* is high either in fresh water or marine *Atherina* spp. This may due to higher prevalence of *S. aureus* in human being either on their skin or in their nose that create a great chance for contamination of *Atherina* species up on improper handling through persons who are not observing the basic rules of personal hygiene. This substantiates the conclusion of Papadopoulou *et al*. (2007) that the origin of *S. aureus* is the handlers rather than fish.

Coliforms are commonly inhabitant in the intestinal tract of animals and human and its presence in fish may be attributed to absence of acceptable hygienic measures during harvesting or to sewage contamination of the aquatic environment of fish (Mona *et al*., 2003). The flesh of healthy fish is considered bacteriologically sterile (National Academy of Science, 1985). The bacterial isolates from gills and skin could be mainly accounted for filtering ability of the gills or the slime layer of the skin and particularly as a result of active multiplication and adaptation. Such bacteria may spread to the vascular system and invade the fish flesh, which are not in direct contact with the external aquatic environment (Shewan, 1971).

Table (1): Occurrence of *Staphylococcus spp*. in surface swabs and muscle samples of *Atherina* fish.

Isolated microorganisms	*Atherina* spp								Total number (300)			
	Fresh water *Atherina* spp (210)				Marine *Atherina* spp (90)							
	SU.S		Ms		SU.S		Ms		SU.S		Ms	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
S. aureus	138	65.7	75	35.7	56	62.2	23	25.6	194	64.7	98	32.7
Coagulase negative *Staphylococcus* spp	82	39.1	55	26.2	38	42.2	11	12.2	120	40	66	22

SU.S: Surface swabs of fish MS: Muscle samples of fish No.: Number of positive

The results of Table (2) verify that the isolated *Enterobacteriaceae* were *E. coli, Kl. oxytoca, Kl. pneumoniae, Ent. cloacae, P. vulgaris, P. mirabilis, Sh. sonnei, Cit. freundii, Cit. koseri, Pantoea agglomerans, Hafnia alvei, M. morganii* and unidentified microorganisms. The respective overall prevalence of those pathogens in surface swabs versus muscle samples of the all examined *Atherina* spp were 5.33% Vs 0.7%, 7% Vs 2.3%, 5.7% Vs 1.3%, 5% Vs 1.7%, 9% Vs 5%, 6.3% Vs 3.7%, 1.7% Vs 0.7%, 5% Vs 1.7%, 6% Vs 2.2%, 38.3% Vs 24.7%, 1.7% Vs 1.3%, 2.3% Vs 1.3%, and 8.7% Vs

3.7%, respectively. Nearly similar results were obtained by Abo El-Alla and Bastawrows (1999), who isolated *Kl. pneumoniae* (0.58%), *Kl. oxytoca* (1.73%), *Cit. freundii* (10.14%), *P. vulgaris* (1.73%), and *P. mirabilis* (8.41%) from *Tilapia nilotica*. They also isolated these pathogens with respective percentages of 0.56, 2.81, 7.87, 5.16 and 6.74 from *Labeo niloticus.* However, all samples they examined were free from *E.coli*.

On the other hands, Maysa and Abd Elall (2009) isolated the same pathogens from *Tilapia nilotica* and *Clarias lazera*, their results were *E. coli* (30%), *Ent. cloacae* (20%), *Ent. agglomerans* (8.33%), *Ent. aerogens* (3.33%), *Cit. freundii* (13.33%), *Kl. pneumoniae* (16.67%), *Kl. oxytoca* (8.33%), *P. vulgaris* (21.67%), and *P. mirabilis* (8.33%). Moreover, Sifuna *et al.*, (2008) found that all the examined fish samples (100%) were positive for presence of *E. coli*. They concluded that the pollution of river's and lakes' water by products of man continues to create public health problem especially food borne diseases caused by members of family *Enterobacteriaceae.*

Results of (Table 2) also verify that *Enterobacteriaceae* isolated from the surface swabs versus muscle samples of fresh water *Atherina* fish were *E.coli* 5.71% Vs 0.95%, *Kl. oxytoca*, 8.1% Vs 2.4%, *Kl. pneumoniae* 6.7% Vs 1.4%, *Ent. cloacae* 6.2% Vs 1.9%, *P. vulgaris* 9.5% Vs 5.7%, *P. mirabilis* 6.7% Vs 4.3%, *Sh. sonnei* 1.9% Vs 0.95%, *Cit. freundii* 5.71% Vs 1.9%, *Cit. koseri* 6.2% Vs 2.9%, *Pantoea agglomerans* 38.1% Vs 26.2%, *Hafnia alvei* 1.9% Vs 1.4%, *M. morganii* 2.4% Vs 1.4%, and unidentified microorganisms 6.7% Vs 4.3%.

On the other hand, the respective occurrence of the aforementioned pathogens in surface swabs versus muscle samples of marine *Atherina* were 4.4% Vs 0%, 4.4% Vs 2.2%, 3.3% Vs 1.1%, 2.2% Vs 1.1%, 7.8% Vs 3.3 %, 5.6% Vs 2.2%, 1.1% Vs 0%, 3.3% Vs 1.1%, 5.6% Vs 2.2%, 38.89% Vs 21.1%, 1.1% Vs 1.1%, 2.2% Vs 1.1% and 13.3% Vs 2.2%. Higher percentages of the isolated pathogens were previously reported by Papadopoulou *et al.* (2007). The highest infection rate may be attributed to unsatisfactory sanitation during handling and processing.

From Table (2) it could be easily concluded that the presence of *Enterobacteriaceae* in fish may be related to fecal pollution of surface water or aquatic environment of fish or to improper handling. From zoonotic point of view, it constitutes a public health hazard.

Table (2): Occurrence of *Enterobacteriaceae* in surface swabs and muscle samples of *Atherina* fish.

Isolated microorganisms	*Atherina* spp. A								Total number (300)			
	Fresh water *Atherina* spp. (210)				Marine *Atherina* spp. (90)							
	SU.S		MS		SU.S		MS		SU.S		MS	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
E. coli	12	5.71	2	0.95	4	4.4	0	0	16	5. 33	2	0.7
Kl. oxytoca	17	8.1	5	2.4	4	4.4	2	2.2	21	7	7	2.3
Kl. pneumoniae	14	6.7	3	1.4	3	3.3	1	1.1	17	5.7	4	1.3
Ent. cloacae	13	6.2	4	1.9	2	2.2	1	1.1	15	5	5	1.7
P. vulgaris	20	9.5	12	5.7	7	7.8	3	3.3	27	9	15	5
P. mirabilis	14	6.7	9	4.3	5	5.6	2	2.2	19	6.3	11	3.7
Sh. Sonnei	4	1.9	2	0.95	1	1.1	0	0	5	1.7	2	0.7
Cit. freundii	12	5.71	4	1.9	3	3.3	1	1.1	15	5	5	1.7
Cit. koseri	13	6.2	6	2.9	5	5.6	2	2.2	18	6	8	2.2
Pantoea agglomerans	80	38.1	55	26.2	35	38.89	19	21.1	115	38.3	74	24.7
Hafnia alvei	4	1.9	3	1.4	1	1.1	1	1.1	5	1.7	4	1.3
M. morganii	5	2.4	3	1.4	2	2.2	1	1.1	7	2.3	4	1.3
Unidentified microorganisms	14	6.7	9	4.3	12	13.3	2	2.2	26	8.7	11	3.7

SU.S: Surface swabs of fish. MS: muscle samples of fish No.: Number of positive.

Table (3) shows that *S. aureus* and coagulase negative *Staphylococcus* spp were isolated from 69 (53.1%) and 55 (42.3%) of the examined water samples, respectively. Lower percentages of 20, and 10 were previously recorded by Mohamed *et*

al. (2003), and Metawea and Abd El-Ghaffar (2007), respectively.

The highest percentages of *S. aureus* and coagulase negative *Staphylococcus* spp. may be attributed to contamination of the hands of *Atherina* fish handlers which in turn contaminates *Atherina* fish and water used for their preparation for selling. It was clarified by Le-Loir *et al.* (2003), that *S. aureus* is a main cause of gastroenteritis resulting from the consumption of contaminated food. Information from many episodes of Staphylococcal gastroenteritis outbreaks indicates that *S. aureus* was isolated from the implicated food and from the hands and nose of food handlers (Docarmo *et al.*, 2004). On the other hand, coagulase negative *Staphylococcus* spp. was reported to produce enterotoxin and affect the public health (Udo *et al.*, 1999).

Regarding the occurrence of *Staphylococcus* spp. in hand swabs of *Atherina* fish handlers; table (3) clarifies that S. *aureus* was isolated from 73% of hand swabs. Nearly similar results were previously reported by Lues and Tonder (2007) and Simon and Sanjeev (2007). Lower frequencies were also cited by Mohamed *et al.* (2001), Shojaei *et al.* (2006), Dhorod *et al.* (2009) and Ibrahim *et al.* (2009).

On the other hand, high percentage of 95 was recorded by Aycicek *et al.* (2004). The variation between the obtained results and the previous studies may be due to geographical distribution, the extent of applying the personal hygiene and the time of sampling.

Table (3) shows also that the percentage of caogulase negative *Staphylococcus* spp. in hand swabs was 50. Higher percentage of 70 was obtained by Aycicek *et al.* (2004). However, lower result was previously recorded by Mohamed *et al.* (2001).

The higher percentage of *S. aureus* (73) and caogulase-negative *Staphylococcus* spp. (50) from the hand swabs of *Atherina* fish handlers can be explained by the fact that these organisms are found as a permanent flora on the skin. It was previously reported that approximately 35-40% of the healthy adults carry *S. aureus* asymptomatically either on their skin or mucosa (Ulrich, 1965). So, it could be easily concluded that the fish handlers are still a potential hazard for Staphylococcal food contamination.

Table (3): Occurrence of *Staphylococcus* spp in hand swabs of *Atherina* fish handlers (n=100) and water used for preparation of *Atherina* spp for selling (n=130).

***Staphylococcus* species isolate**	**hand swabs of Atherina fish handlers (n=100)**		**Water used for preparation of Atherina spp for selling (n=130)**	
	No. of +ve	**%**	**No. of +ve**	**%**
S. aureus	73	73	69	53.1
Coagulase negative *Staphylococcus spp*	50	50	55	42.3

The results in Table (4). The table shows that the isolated *Enterobacteriaceae* from water samples were *E. coli* 8 (6.15%), *P. vulgaris* 15 (11.5%), *P. mirabilis* 10 (7.7%), *Ent. cloacae* 10 (7.7%), *Cit. freundii* 8 (6.15%), *Cit. koseri* 9 (6.9%), *Kl. pneumoniae* 10 (7.7%), *Kl. oxytoca* 12 (9.2%), *Pantoea agglomerans* 40 (30.7%), *Hafnia alvei* 3 (2.3%), *M. morganii* 4 (3.1%), and *Sh. sonnei* 2 (1.5%). Nearly similar results were cited by Zaki (1989), Metawea and Abd El-Ghaffar (2007), and Maysa and Abd El-All (2009).

The variation in the percentages of the isolation rates of *Enterobacteriaceae* between this study and others may be attributed to the type of water examined. In this study, the water samples examined were tape water in which *Atherina* spp were immersed for preparation for selling, however, water samples examined in other studies were surface water from rivers or seas into which large quantities of sewage and animal wastes may be dumped.

It could be easily concluded from table (4) that water might play an important role as a reservoir and a source of human infection with *Enterobacteriaceae*. The presence of such pathogens in water may be a result of fecal contamination of water by dirty hands of *Atherina* fish handlers, who are not observing the basic rules of personal hygiene especially after using toilet. Also it throw light on the role of *Atherina* in transmitting pathogenic bacteria to man.

Table (4) also points to the isolation of *E. coli, P. vulgaris, P. mirabilis, Kl. pneumoniae, Kl .oxytoca, Ent. cloacae, Cit. freundii, Cit. koseri, Pantoea agglomerans and unidentified* spp. with the percentages of 5, 8, 5, 6, 7, 6, 4, 5, 36, and 18, respectively. These results agree with Maysa and Abd-Elall (2009) and Shojaei *et al.* (2006).

From the results illustrated in table (4), one can easily concluded that the isolation of *E .coli, Ent. cloacae, P. vulgaris, P .mirabilis, Kl. pneumoniae , Kl. oxytoca, Cit. freundii, Cit. koseri and Pantoea agglomerans* from hands of *Atherina* fish handlers were due to faecal to hand spread with no application of the basic rules of personal hygien. This was previously verified by Ampofo and Clerk (2002), who reported that the isolation of *E. coli, Kl .pneumoniae, P. mirabilis and P. vulgaris* were common in individuals of communities of sewage fed

ponds. De Wit and Rombouts (1992) concluded that the presence of *Enterobacteriaceae* on hands is not a good indicator of personal and toilet hygiene.

From the aforementioned results, it is evident that *Enterobacteriaceae* are potentially present in water and are not known as classical fish pathogens. The oxygen depletion and higher water temperature render fishes to be easily infected with those bacteria (Badran *et al.,* 1994). Moreover, fish might act as a carrier of human pathogens such as *Staphylococcus* species and members of *Enterobacteriaceae* in water environments polluted by human sewage or diseased animal. Therefore, good water quality is the key to improve the production and hygiene of fish as a food (Omaima and El-Kewaley, 2008).

Table (4): Occurrence of *Enterobacteriaceae* in hand swabs of *Atherina* fish handlers (n=100) and water used for preparation of *Atherina* spp for selling (n=130).

Isolated microorganisms	Hand swabs of Atherina fish handlers (n=100)		Water used for preparation of Atherina spp for selling (n=130)	
	No. of +ve	%	No. of +ve	%
E. coli	5	5	8	6.15
P. vulgaris	8	8	15	11.5
P. mirabilis	5	5	10	7.7
Ent. cloacae	6	6	10	7.7
Cit. freundii	4	4	8	6.15
Cit. koseri	5	5	9	6.9
Kl. pneumoniae	6	6	10	7.7
Kl. oxytoca	7	7	12	9.2
Pantoea agglomerans	36	36	40	30.7
Hafnia alvei	0	0	3	2.3
M. morganii	0	0	4	3.1
Sh. Sonnei	0	0	2	1.5
Unidentified microorganism	18	18	5	3.8

Results in Table (5) reveal that O_{128} (2 strains), O_{114} (1 strain), and O_{136} (1 strain) were isolated from *Atherina*. However, O_{26}, O_{111}, and untyped strain (1 strain, each) were identified in hand swabs of *Atherina* fish handlers. While, the 3- *E. coli* isolates from water samples used for preparation of *Atherina* fish for selling were O_{128}.

It was clear from table (5) that the most predominant serogroup of *E. coli* was O_{128} which isolated from *Atherina* spp. and water samples. Hefnawy *et al.* (1989) isolated enteroopathogenic *E. coli* from *Tilapia nilotica* and their serogrouping were O_{55}, O_{86a}, O_{128a}, O_{128b} (one strain, each) and O_{119} (2 strains). On the other hand, Metawea and Abdel Ghaffar (2007) identified *E. coli* O_{55}: K_{59}, O_{124}: K_{72}, O_{111}: K_{55}, and O_{128}: K_{67} from fish samples and their aquatic environment. They concluded that the presence of the same serotypes in both water and fish samples indicates that water may act as a dangerous source of these pathogens to fish and consequently act as a vehicle of human infection, constituting therefore public health problems.

O_{136} is one of the enteroinvasive *E. coli* (EIEC) that invades and multiplies with in human colonic epithelial cells. Human is its natural reservoir. Person to person transmission through fecal-oral route and ingestion of contaminated food and water that have been contaminated through dirty hands of fish or food handlers who are not observing the basic rules of personal hygiene are the principal modes of its transmission (Meng *et al.*, 2001).

The isolation of O_{136} in *Atherina* fish may arise from pollution of aquatic environment with sewage or from dirty hands of fish handlers. This result substantiates the role of water and fish handlers in transmission of pathogens to fish which in turn transmit these pathogens to other susceptible people through ingestion of improperly cooked fish or through direct contact.

Microorganisms differ in their response to freezing, some survive virtually unharmed, some resist freezing but are susceptible to damage during frozen storage (Hossain *et al.,* 2008). The damage caused to microorganism by freezing was due to sudden drop in temperature, ice crystals formation, and increase of solutes concentration (Lund, 2000).

Table (5): Serogrouping of *E. coli* isolates from *Atherina* spp., water used for preparation of *Atherina* spp. for selling and their handlers.

E. coli serotypes	Source			Total
	Atherina species	water used for preparation of *Atherina* species for selling	Hand swabs of *Atherina* fish handlers	
O26	0	0	1	1
O111	0	0	1	1
O114	1	0	0	1
O128	2	3	0	5
O136	1	0	0	1
Untyped	0	0	1	1
Total	4	3	3	10

Table (6) shows that after the 1st week from freezing, all *Enterobacteriacea* detected before freezing were continued to isolate with a ratio of (1:4, each) from the surface swabs of the four examined *Atherina*. On the other hand, *S. aureus* and coagulase negative *Staphylococcus* spp were continued to recover from surface swabs with ratios of (4:4), and (2:4), respectively.

All *Enterobacteriaceae* except *P. mirabilis* (each with a ratio of 1:4), *S. aureus* (4:4), and coagulase negative *Staphylococcus* spp (1:4) were continued to isolate from surface swabs after the 2nd week from freezing. On the other hand, the isolated species after the 3rd week of freezing were *Kl. oxytoca* and *Pantoea agglomerans* (1:4, each), and *S. aureus* (4:4). Meanwhile, after the 4th week of freezing, the isolated species were *Pantoea agglomerans (1:4)* and *S. aureus* (4:4). *S. aureus* (4:4) was found positive until the 13th week from freezing. In contrary, all muscle samples were negative since the 1st week of freezing.

These results agree with Chattopadhyay (1999), Lund (2000), and Hossain *et al.* (2008), who stated that gram negative bacteria are more sensitive to freezing than gram positive bacteria. Thushani *et al.* (2003) also concluded that *S. aureus* survived during storage at -24°C over a period of 8 weeks. The resistance of *S. aureus* to freezing may be related to the presence of mucoprotein complex and diaminopimelic acid in the cell walls of gram positive cells. The mucoprotein complex and diaminopimelic acid was found to protect the membrane protein against denaturation (Singhal and Pushpa, 1999). From hygienic point of view, freezing at -20°C for suitable time prevent transmission of some pathogenic bacteria to man through eating *Atherina* spp.

Salting of fish is an ancient method used by the Pharos for preservation of fish. The effect of salting on the microorganisms arise from the changes of the osmotic pressure which cause lowering the moisture content of the microbial cell, leading to its shrinkage and death. Moreover, salting cause denaturation of the protein content of the microbial cell, this considered as an important factor in suppression of the microbial growth (Ez El-Din, 1999).

Table (6): The effect of freezing on the survival of *Staphylococcus spp*. and *Enterobacteriaceae* in fresh water *Atherina* fish.

Isolated Bacteria	Before treatment *				After freezing**												
	SU.S		MS		1w	2w	3w	4w	5w	6 w	7 w	8 w	9 w	10 w	11 w	12 w	13 w
	No.	%	No.	%	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.
Kl. oxytoca	2	20	1	10	1	1	1	0	0	0	0	0	0	0	0	0	0
P. vulgaris	1	10	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
P. mirabilis	1	10	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Pantoea agglomerans	5	50	3	30	1	1	1	1	0	0	0	0	0	0	0	0	0
Cit. freundii	1	10	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Cit. koseri	1	10	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
S. aureus	6	60	4	40	4	4	4	4	4	4	4	4	4	4	4	4	4
Coagulase negative *Staphylococci*	4	40	3	30	2	1	0	0	0	0	0	0	0	0	0	0	0

No. : Number of positive. *Ten *Atherina* fish were examined before treatment

** Surface swabs and muscle samples of 4 frozen *Atherina* fish was examined/week.

-Muscles of all fish examined after the 1st week from freezing treatments were found to be negative.

The results of salting experiment were shown in table (7). The illustrated results verify that the ratios of the isolation of the bacterial species from surface swabs after salting treatment was lower than before treatment. It is also clear from table (7) that *Kl. oxytoca, P. vulgaris, P. mirabilis* (1:4, each) were isolated from surface swabs of fresh water *Atherina* salted in 25% NaCl solution. Moreover, *Pantoea agglomerans* and *S. aureus* were isolated from the same samples with ratios of (2:4) and (4:4), respectively. On the other hand, only *Kl. oxytoca* and *Pantoea agglomerans* were isolated from the muscle samples of *Atherina* fish salted in 25% NaCl solution with a ratio of (1:4, each). Meanwhile, *S. aureus* was isolated from the same samples at a ratio of (2:4).

Table (7) shows also that all samples salted in 50% and 75% NaCl solution were negative for the presence of *Enterobacteriaceae* from the 1st week and for the whole period of the experiment. Moreover, all muscle samples were negative at all dilutions except the dilution of 25%. On the other hand, *S. aureus* was isolated from surface swabs of *Atherina* fish at a rate of (2:4) after 2 and 3 weeks and at a rate of (1:4) after four and five weeks.

The obtained results agree with Ez El-Din (1999), who reported that salting appeared to have a little effect on the growth of aerobic, mesophilic bacteria, *Streptococcus faecalis, S. aureus*, and mold but completely suppressed coliforms growth. Slonczewski *et al.* (2008) also found that *S. aureus* resists salting for long period. He concluded that lower water activity (0.85) of *S. aureus* enable to grow in up to 25% NaCl solution. It is easily concluded from table (7) that salting has a great effect on *Enterobacteriaceae* and other gram negative bacteria and a little effect on *S. aureus.* This may throw light on the cause of food intoxication after consumption of salted fish meal.

Table (7): The effect of salting on the survival of *Staphylococcus* spp. and *Enterobacteriaceae* in fresh water *Atherina* fish.

Bacteria Isolated	Before treatment *				After salting**												
					1w				2w			3w			4w	5w	6w
					25%		50%	75%	25%	50%	75%	25%	50%	75%	25%	25%	25%
	SU.S	MS	SU.S	MS	SU.S	MS	SU.S	SU.S	SU.S	SU.S	SU.S	SU.S	SU.S	SU.S	SU.S	SU.S	SU.S
	No.	%	No.	%	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.
Kl. oxytoca	2	20	1	10	1	1	0	0	0	0	0	0	0	0	0	0	0
P. vulgaris	1	10	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
P. mirabilis	1	10	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Pantoea agglomerans	5	50	3	30	2	1	0	0	0	0	0	0	0	0	0	0	0
Cit. freundii	1	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cit. koseri	1	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. aureus	6	60	4	40	4	2	0	0	2	0	0	2	0	0	1	1	0
Coagulase negative *Staphylococci*	4	40	3	30	0	0	0	0	0	0	0	0	0	0	0	0	0

No. : Number of positive. *Ten *Atherina* fish were examined before treatment

** Surface swabs and muscle samples of 4 frozen *Atherina* fish was examined/week.

- Muscles of all *Atherina* fish salted 50%, 75% NaCl were found to be negative from the 1st week of salting.

The results of commercial vinegar treatment on the survivability of *Enterobacteriaceae* and *Staphylococcuc* spp in Atherina fish are shown in table (8). It is clear that *Pantoea agglomerans* and *Kl. oxytoca* continued to isolate from surface swabs of treated *Atherina* untill 3 hrs after vinegar treatment. On the other hand, *E. coli* continued to isolate with a ratio of (1:4) from the treated *Atherina* until 6 hrs from vinegar treatment. In contrary all treated *Atherina* fish were negative for *S. aureus* since the 1st hour of treatment. These results are in agreement with Entani *et al.* (1998), who reported that the growth of some food borne pathogenic bacteria was inhibited with 0.1% concentration of acetic acid in the used vinegar.

From zoonotic point of view the obtained results illustrated in table (8) revealed that the commercial vinegar is effective for reducing bacterial population in fish due to its content of acetic acid. It was reported by Rhee *et al.* (2003) that the antimicrobial effect of acetic acid on the inactivation of food borne pathogenic bacteria stored at 22°C was more effective than at 5°C. Heat and acetic acid treatments act synergistically to inhibit the growth of food borne pathogens (Shin *et al.*, 2006).

Table (8): The effect of commercial vinegar (5% acetic acid) on the survival of *Staphylococcus* spp. and *Enterobacteriaceae* in fresh water *Atherina* spp. at room temperature

Isolated Bacteria	Before treatment *				After treatment**											
					1 h		2 h		3 h		4 h		5 h		6 h	
	SU.S		MS		SU.S	MS	SU.S	MS	SU.S	MS	SU.S	MS	SU.S	MS	SU.S	MS
	No.	%	No.	%	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.
E. coli	1	10	1	10	1	1	1	1	1	1	1	1	1	1	1	1
P. vulgaris	2	20	1	10	1	1	1	1	0	0	0	0	0	0	0	0
P. mirabilis	1	10	1	10	1	0	0	0	0	0	0	0	0	0	0	0
Pantoea agglomerans	5	50	3	30	2	2	2	1	2	0	0	0	0	0	0	0
Kl. pneumoniae	1	10	1	10	1	0	0	0	0	0	0	0	0	0	0	0
Kl. oxytoca	1	10	1	10	1	1	1	0	1	0	0	0	0	0	0	0
S. aureus	6	60	5	50	0	0	0	0	0	0	0	0	0	0	0	0
Coagulase negative *Staphylococcus* spp	4	40	3	30	0	0	0	0	0	0	0	0	0	0	0	0

No. Number of positive *Ten *Atherina* fish were examined before treatment
**Surface swabs and muscle samples of 4 frozen *Atherina* fish was examined/week.
All treated fish samples examined after 7 h were negative.

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Quality of Life of School Age Thalassemic Children at Zagazig City

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Abstract: Background: The assessment of quality of life in children, especially in those with chronic illness such as thalassaemia, is particularly important. It differs from other forms of medical assessment in that it focuses on the individuals' own views of their well-being and other aspects of life, giving a more holistic view of well-being. The aim of the present study was to: assess the quality of life of school-age children with Thalassemia at Zagazig City. Subjects and Methods: A descriptive study was conducted on a sample of 100 school-age thalassemic children at out-patient Hematology clinic at Zagazig University Hospitals in Sharkia Governorate, Egypt. Two tools were used to collect the necessary data. The first was a structured interview questionnaire sheet including socio-demographic data of children and their parents as well as medical history. The second tool was a standardized tool (the Pediatric Quality of Life Inventory ^{TM} Version 4.0). Results: The results of the present study revealed that the quality of life of school-age children with Thalassemia Major was affected. There was a significant association between the total quality of life and compliance with blood transfusion in both child and parent report. In addition, there was a significant association between the total quality of life and regular iron chelation therapy. Concluosion: Thalassaemia has a negative impact on perceived physical, emotional, social and school functioning in thalassaemia patients. Recommendations: Suitable programs aiming to increase children's adherence to the treatment regimen should be provided to increase psychosocial support.

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Key words: Quality of life, thalassemia major, school-age.

1. Introduction:

Thalassemias are inherited disorders of Hb synthesis that result from an alteration in the rate of globin chain production. A decrease in the rate of production of a certain globin chain or chains (α, β, γ, δ) impedes Hb synthesis and creates an imbalance with the other, normally produced globin chains (Madara & Pomarico-Denino, 2007).

The most severe form is the β-thalassemia major, which is characterized by a severe microcytic, hypochromic anemia (Cooley's anemia), whose symptoms appear usually within the first 2 years of life. Infants become pale and asthenic, have a poor appetite, grow slowly, and often develop jaundice; spleen, liver, and heart may also be enlarged. Adolescents with the most severe form may experience delayed puberty ((Mazzone et al, 2009)).

β-thalassemia is the most common chronic hemolytic anemia in Egypt (85.1%). A carrier rate of 9-10.2% has been estimated in 1000 normal random subjects from different geographical areas of Egypt (El-Beshlawy, 1999).

Children with thalassemia major have good survival but little is known about their quality of life (Sachdeva et al, 2002). Children not only have longer lives to be compared to adults, but they are less able to voice their concerns and are more vulnerable than adults (Ismail et al, 2006).

The assessment of quality of life (QOL) in children especially in children with chronic illness such as thalassaemia is particularly important (Ismail, 2006). An assessment of QOL differs from other forms of medical assessment in that it focuses on the individuals' own views of their well-being and assesses other aspects of life, giving a more holistic view of well-being (Dahlui et al., 2009).

It is important to understand more about quality of life in pediatric population to evaluate and improve the care patients receive. Children with chronic physical illness exemplified thalassemia are vulnerable to emotional and behavioral problems leading to poor quality of their lives. The disease may cause a sense of stigmatization in the child leading to feeling of shame and rejection. It also may affect social relations, school interactions, and self-esteem. So, great attention has to be taken especially by the nurse during follow up and treatment.

The aim of the present study was to

Assess quality of life of school age children with thalassemia.

2. Subjects and Methods:

Research design

A descriptive study was conducted to assess the quality of life of school-age children with thalassemia major.

Research Question

What is the impact of thalassemia as a chronic disease on the quality of life of school-age children?

I- Subjects

1. Setting

The study was conducted at Out-Patient Hematology Clinic at Zagazig University Hospitals in Sharkia Governorate.

2. Subject

The study was conducted on a sample of 100 school-age children who fulfilled the following criteria:-

1- Confirmed diagnosis of thalassemia major.
2- Age: from 6 years old to 12 years old
3- Both sexes.
4- Free from any other chronic diseases.

3. Tools for data collection

Two tools were used to collect the necessary data.

Tool I: Structured interview questionnaire sheet

Structured interview questionnaire sheet was developed by the researcher to collect the following necessary data:

A- Biosocial data of the child as, child's age, sex, birth date, and level of education.
B- Information about disease history, any similar conditions in the family, number of blood transfusions per month, compliance with blood transfusion and compliance with chelation therapy.
C- Socio-demographic data of child's parents, such as, age, educational level, occupation, family income as well as crowding index.

Tool Π: The Pediatric Quality of Life Inventory™ Version 4.0 by Varni et al., 1998.

The Peds QL™ Measurement Model is a modular approach to measure health-related quality of life (HRQOL) in healthy children and adolescents and those with acute and chronic health conditions. The Peds QL™ Measurement Model integrates seamlessly both generic core scales and disease-specific modules into one measurement system.

This form includes:

- Young children report (ages 5-7)
- Parent report for young children (ages 5-7)
- Child report (ages 8-12)
- Parent report for children (ages 8-12)

There are four domains in each report. The PedsQL version 4.0 consists of 23 items including the following:

1) Physical functioning (eight items)
2) Emotional functioning (five items)
3) Social functioning (five items)
4) School functioning (five items)

Scoring system for assessment of the quality of life (Varni et al., 1998):

The Peds QLTM 4.0 Generic Core Scales are comprised of parallel child self-report and parent proxy-report formats. Child self-report includes ages 5-7, and 8-12 years. Parent proxy-report includes ages 5-7 (young child), and 8-12 (child), and assesses parent's perceptions of their child's HRQOL. The instructions ask how much of a problem each item has been during the past 1 month.

0 = I never have a problem
1 = I almost never have a problem
2 = I sometimes have a problem
3 = I often have a problem
4 = I almost always have a problem.

Π- Methods

1- An official permission was obtained to facilitate collection of data.
2- Sociodemographic questionnaire sheet was developed by the researchers after thorough review of literature.
3- Jury was done to the tool by 5 experts (Three professors of pediatric nursing, one professor of hematology, and one professor of statistics).
4- The Pediatric Quality of Life InventoryTM Version 4.0 by Varni et al., 1998.
5- Written consent was obtained from parents of children.
6- A pilot study was conducted on 10% of children and their parents to test the clarity of questions and to estimate the time required for filling the sheet and no modification was done.
7- Each child and his/her mother were individually interviewed to collect the necessary data using tool I and tool Π. The time consumed to answer the questionnaire sheet ranged from 25 to 30 minutes. The average number of children and their mothers/day was 6 children.
8- Data was collected during 10 months, starting from July 2008 to April 2009.

Statistical analysis

The collected data was coded and entered in a data base file using the Foxpro for windows program. After complete entry, data were transferred to the SPSS version 14.0 program by which the analysis was conducted applying frequency tables with percentages and cross tabulations. The chi-square test was used to find the significant associations between the demographic and clinical data and the outcome measures.

3. Results

Table (1) shows the characteristics of the studied thalassemic children. Regarding the children's age, 24% were from 6 to 7 years. While 76% were aged from 8 to 12 years with mean age of 9.29±2.17 years. Also, 56% were males and 44% were females. Those who ranked the first birth order constituted 28%, the second 25%, while 24% were the third. Only 8% were either the fifth or more in birth order. It is revealed from the same table that 76% were from rural compared to 24% were from urban.

Regarding family income, 20% of the studied children's families had insufficient income, 69% had slightly sufficient income. On the other hand, 11% had sufficient and save income. As regard to crowding index, 37% of families of the studied children were with crowding index of < 2, while 55% were with crowding index of 2-3, as well as 8% only had crowding index of >3 .

Table (1): Characteristics of the Studied Thalassemic Children

Characteristics	No. (100)	%
Age in years:		
6-7 years	24	24.0
8-12 years	76	76.0
Mean ± SD	**9.29±2.17**	
Sex:		
Male	56	56.0
Female	44	44.0
Birth order:		
The first	28	28.0
The second	25	25.0
The third	24	24.0
The fourth	15	15.0
The fifth and more	8	8.0
Residence:		
Rural	77	77.0
Urban	23	23.0
Crowding index		
< 2	37	37.0
2-3	55	55.0
> 3	8	8.0
Family income :		
Insufficient	20	20.0
Slightly sufficient	69	69.0
Sufficient and save	11	11.0

Table (2) shows the medical history of the studied thalassemic children. It reveals that children who were diagnosed as β-thalassemia major by the first year of life constituted 83%, while 17% only were diagnosed by the second year. Consanguinity was found among 65% of the parents, while 35% of them negative consanguinity. It was also found that 57% had similar conditions in the family and 43% had no conditions.

Regarding compliance with treatment, 48% of the studied children had irregular blood transfusion therapy, while 52% were regulars. In addition, 42% of the studied children were compliant with iron chlation therapy, while 58% were not compliant.

It was revealed from the same table that, 68% of the studied children come to the hospital once per month for transfusion therapy, while 32% come twice per month.

Table 3 portrays the total QOL score and its domains. According to child's report, 37% of children had good score compared to 21% in parent's report, while 58% had fair score compared to 64% in parent's report.

Table (2): Medical History of the Studied Thalassemic Children

Medical History	No. (100)	%
Disease occurrence:		
By 1st year	83	83.0
By 2nd year	17	17.0
Consanguinity:		
Present	65	65.0
Not present	35	35.0
Similar conditions in the family:		
Present	57	57.0
Not present	43	43.0
Transfusion therapy:		
Regular	52	52.0
Irregular	48	48.0
Iron chelation therapy:		
Regular	42	42.0
Irregular	58	58.0
No. of transfusion therapy/month:		
Once	68	68.0
Twice	32	32.0

Table (3): The Total QOL Scale and QOL Subscales of the Studied Thalassemic Children

QOL scale	*Very good* =100%		Good 75-99.9		Fair 50-74.9		Bad 25-49.9		Very bad 0-24.9		Mean ± SD
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	
Child report:											
Total QOL	0	0.0	37	37.0	58	58.0	5	5.0	0	0.0	73.1±12.4
Physical	3	3.0	56	56.0	39	39.0	2	2.0	0	0.0	63.1±17.8
Emotional	1	1.0	28	28.0	47	47.0	21	21.0	3	3.0	60.2±20.1
Social	5	5.0	57	57.0	30	30.0	7	7.0	1	1.0	84.15±12.4
School	2	2.0	35	35.0	46	46.0	17	17.0	0	0.0	74.95±16.5
Parent's report:											
Total QOL	0	0.0	21	21.0	64	64.0	15	15.0	0	0.0	64.8±13.8
Physical	1	1.0	25	25.0	48	48.0	22	22.0	4	4.0	60.62±19.7
Emotional	0	0.0	23	23.0	43	43.0	27	27.0	7	7.0	54.8±21.14
Social	4	4.0	57	57.0	28	28.0	10	10.0	1	1.0	73.05±17.8
School	4	4.0	33	33.0	47	47.0	15	15.0	1	1.0	66.55±17.1

Regarding physical QOL, 56% had good score according to child's report compared to 25% in parent's report, while 39% had fair score compared to 48% in parent's report.

In relation to emotional QOL, 28% had good score according to child's report compared to 23% in parent's report. On the other hand, 21% had bad score compared to 27% in parent's report, while 47% had fair score compared to 43% in parent's report.

Regarding social QOL, 57% had good social score in both child and parent's report, while 30% had fair social score compared to 28% in parent's report.

Regarding school QOL, 35% had good score according to child's report compared to 33% in parent's report. Forty six percent of children had fair score compared to 47% in parent's report while, 17% of children had bad score compared to 15% in parent's report.

The same table reveals that the emotional functioning scored the lowest followed by physical then school and social functioning according to both child and parent's report.

The QOL scores of the studied thalassemic children regarding the physical and emotional functions are illustrated in table 4. In the child's report, it was found that 79% of children had very good score regarding taking a bath alone compared to 76% in parent's report. As regard walking, according to child's report, 66% had very good score compared to 57% in parent's report.

Table (4): Distribution of the Physical and emotional Functioning of the Studied Thalassemic children

Physical and Emotional domains	Very good		Good		Fair		Bad		Very bad		Total
	N	%	N	%	N	%	N	%	N	%	
Physical domain											
Child report:											
Walking	66	66.0	1	1.0	26	26.0	2	2.0	5	5.0	100
Running	18	18.0	0	0.0	25	25.0	12	12.0	45	45.0	100
Making activity	32	32.0	3	3.0	44	44.0	11	11.0	10	10.0	100
Lifting something	43	43.0	7	7.0	28	28.0	13	13.0	9	9.0	100
Taking a bath	79	79.0	0	0.0	12	12.0	4	4.0	5	5.0	100
Doing chores	51	51.0	12	12.0	28	28.0	6	6.0	3	3.0	100
No pain	31	31.0	0	0.0	49	49.0	14	14.0	6	6.0	100
Having energy	20	20.0	3	3.0	39	39.0	18	18.0	20	20.0	100
Parent report:											
Walking	57	57.0	5	5.0	25	25.0	9	9.0	4	4.0	100
Running	17	17.0	1	1.0	28	28.0	17	17.0	37	37.0	100
Making activity	36	36.0	4	4.0	41	41.0	10	10.0	9	9.0	100
Lifting something	38	38.0	4	4.0	34	34.0	14	14.0	10	10.0	100
Taking a bath	76	76.0	4	4.0	11	11.0	4	4.0	5	5.0	100
Doing chores	46	46.0	11	11.0	32	32.0	7	7.0	4	4.0	100
No pain	21	21.0	1	1.0	51	51.0	22	22.0	5	5.0	100
Having energy	16	16.0	3	3.0	30	30.0	26	26.0	25	25.0	100
Emotional domain											
Child report:											
Being afraid	46	46.0	2	2.0	23	23.0	7	7.0	22	22.0	100
Being sad	38	38.0	3	3.0	44	44.0	9	9.0	6	6.0	100
Being angry	14	14.0	0	0.0	22	22.0	16	16.0	48	48.0	100
sleeping trouble	47	47.0	2	2.0	31	31.0	13	13.0	7	7.0	100
Being worried	65	65.0	6	6.0	15	15.0	10	10.0	4	4.0	100
Parent's report:											
Being afraid	44	44.0	2	2.0	18	18.0	13	13.0	23	23.0	100
Being sad	37	37.0	2	2.0	34	34.0	16	16.0	11	11.0	100
Being angry	8	8.0	1	1.0	14	14.0	19	19.0	58	58.0	100
Sleeping trouble	42	42.0	5	5.0	29	29.0	17	17.0	7	7.0	100
Being worried	56	56.0	6	6.0	16	16.0	13	13.0	9	9.0	100

On the other hand, 45% of the studied thalassemic children had bad score regarding running and no one had very bad score according to child's report, while in parent's report, 17 % had bad score and 37% had very bad score.

Regarding having energy, 20% had very bad score according to child's report compared to 25% in parent's report. Thirty nine percent of children had fair score compared to 30% in parent's report.

As regard activities and exercises, according to child's report, 32% had very good score and 44% had fair score. In comparison with parent's report, 36% had very good score and 41% had fair score.

According to child's report, 43% had very good score regarding lifting something heavy and 9% had very bad score, while in parent's report 38% had very good score and 10% had very bad score .

Regarding pain, 49% had fair score and 14% had bad score according to child's report. While in

parent's report, 51% had fair score and 22% had bad score.

Among studied thalassemic children, 51% had very good score regarding doing chores around the house in child's report compared to 46% in parent's report and 28% had fair score compared to 32% in parent's report.

According to child's report, it was found that 46% of children had very good score regarding fear compared to 44% in parent's report. Those who had very bad score constituted 22% compared to 23% in parent's report.

Regarding sadness, 44% of children had fair score compared to 34% in parent's report. In addition, 38% had very good score compared to 37% in parent's report.

As regard anger, 48% of children had very bad score compared to 58% in parent's report. Only 14% had very good score compared to 8% in parent's report.

In relation to having sleeping trouble, 47% of children had no sleeping trouble and had very good score compared to 42% in parent's report. Children who had fair score constituted 31% compared to 29% in parent's report.

Regarding worry, 65% of the studied children had very good score as they had no worry about what will happen to them compared to 56% in parent's report. Ten percent of children had bad score compared to 13% in parent's report.

Table 5 illustrated the social and school functioning of the studied thalassemic children. According to child's report, it was found that 90% had no problems with getting along with other kids so they had very good score compared to 85% in parent's report. Children who had very good score regarding acceptance of them from other kids as friends constituted 72% compared to 75% in parent's report.

Table (5):Distribution of the Social and School Functioning of the Studied Thalassemic Children

Social and School domains	Very good		Good		Fair		Bad		Very bad		Total
	N	%	N	%	N	%	N	%	N	%	
Social Functioning											
Child's report:											
Getting along with kids	90	90.0	0	0.0	2	2.0	4	4.0	4	4.0	100
Other kids refuse him	72	72.0	0	0.0	20	20.0	4	4.0	4	4.0	100
Teasing from other kids	79	79.0	0	0.0	11	11.0	5	5.0	5	5.0	100
Can't do things as others	41	41.0	3	3.0	44	44.0	3	3.0	9	9.0	100
Keeping up when play	29	29.0	1	1.0	27	27.0	19	19.0	24	24.0	100
Parent report:											
Getting along with kids	85	85.0	0	0.0	5	5.0	4	4.0	6	6.0	100
Other kids refuse him	75	75.0	0	0.0	16	16.0	5	5.0	4	4.0	100
Teasing from other kids	74	74.0	0	0.0	14	14.0	9	9.0	3	3.0	100
Can't do things as others	38	38.0	4	4.0	42	42.0	6	6.0	10	10.0	100
Keeping up when play	26	26.0	2	2.0	26	26.0	21	21.0	25	25.0	100
School Functioning											
Child's report:											
Pay attention in class	58	58.0	3	3.0	29	29.0	7	7.0	3	3.0	100
Forgetting things	71	71.0	1	1.0	21	21.0	5	5.0	2	2.0	100
School work	84	84.0	1	1.0	13	13.0	1	1.0	1	1.0	100
Missing school due to illness	48	48.0	4	4.0	23	23.0	18	18.0	7	7.0	100
Missing school to go to hospital	5	5.0	1	1.0	13	13.0	8	8.0	73	73.0	100
Parent's report:											
Pay attention in class	60	60.0	3	3.0	25	25.0	9	9.0	3	3.0	100
Forgetting things	72	72.0	2	2.0	16	16.0	7	7.0	3	3.0	100
School work	85	85.0	1	1.0	13	13.0	1	1.0	0	0.0	100
Missing school due to illness	46	46.0	5	5.0	19	19.0	23	23.0	7	7.0	100
Missing school to go to hospital	7	7.0	0	0.0	12	12.0	8	8.0	73	73.0	100

Children who were not teased from others and had very good score constituted 79% that compared to 74% in parent report.

In relation to the ability of doing things as other kids, 41% had very good score compared to

38% in parent's report. Children had fair score rated 44% compared to 44% in parent's report.

Regarding keeping up with other kids when playing, 29% of children had very good score compared to 26% in parent's report, while 24% of children had very bad score compared to 25% in parent's report.

Regarding paying attention in class, according to children report it was shown that 58% of children had very good score compared to 60% in parent's report. Children who had fair score constituted 29% compared to 25% in parent's report.

As regard to forgetting things, 72% of children had very good score and the same percent was in parent's report. Regarding keeping up with schoolwork, 84% of children had very good score compared to 85% in parent's report.

In relation to missing school due to illness, 48% of children had very good score compared to 46% in parent's report and 18% had bad score compared to 23% in parent's report. Regarding missing school to go to hospital, 73% of children had very bad score, which was similar to parent's report.

The relation between the total QOL of the studied thalassemic children and number of blood transfusion per month is illustrated in table (6). It was found that 68% of children had blood transfusion once per month, while 32% had blood transfusion twice per month.

Table (6) Relation between the Total QOL of the Studied Thalassemic Children and Number of Blood Transfusion per Month

QOL scale	No. of blood transfusion / month				X^2	P-value
	Once/ month n = 68		*Twice/month* n = 32			
	No.	%	No.	%		
Child's report:						
Good	22	32.4	15	46.9	2.09	.35
Fair	42	61.8	16	50		
Bad	4	5.8	1	3.1		
Parent's report:						
Good	13	19.1	8	25.0	.57	.75
Fair	44	64.7	20	62.5		
Bad	11	16.2	4	12.5		

According to child's report, 46.9% of children who had blood transfusion twice per month had good QOL compared to 32.4% of children who had blood transfusion once per month. Children who had fair score in relation to having blood transfusion once per month constituted 61.8% compared to 50% of children who had twice per month.

According to parent's report, 25% of children who had blood transfusion twice per month had good score compared to 19.1% of children who had blood transfusion once per month. Children who had blood transfusion once per month and had fair score amounted up to 64.7% compared to 62.5% of children who had twice per month and had fair score.

This table shows no statistical significant difference in both child and parent's report between the total QOL and number of blood transfusion per month.

The relation between the total QOL of the studied thalassemic children and their compliance with blood transfusion therapy is illustrated in table (7). It was found that 52% of children were compliant with blood transfusion therapy, while 48% were not compliant.

Table (7) Relation between the Total QOL of the Studied Thalassemic Children and Compliance with Blood Transfusion

QOL scale	Compliance with blood transfusion				X^2	P-value
	Yes n = 52		No n = 48			
	No.	%	No.	%		
Child's report:						
Good	27	51.9	10	20.8	11.19	.004*
Fair	24	46.2	34	70.8		
Bad	1	1.9	4	8.3		
Parent's report:						
Good	16	30.8	5	10.4	7.3	.03*
Fair	31	59.6	33	68.8		
Bad	5	9.6	10	20.8		

According to child's report, 51.9% of compliant children had good QOL compared to 20.8% of children who were not compliant. Among compliant children 46.2% had fair score compared to 70.8% of not compliant children. On the other hand, 2.4% of compliant children had bad QOL compared to 6.4% of not compliant children

According to parent's report, 30.8% of compliant children had good QOL compared to 10.45% of not compliant children. Compliant children who had fair score constituted 59.6% compared to 68.8% of not compliant children and 9.6% of compliant children had bad QOL compared to 20.8% of not compliant children.

The table revealed that there was statistical significance between the total QOL of the studied thalassemic children and compliance with blood transfusion according to both child and parent's report (P value is statistically significant at < 0.05).

The relation between the total QOL of the studied thalassemic children and compliance with chelation therapy is illustrated in table (8). It was found that 41% of children were compliant with chelation therapy, while 59% were not compliant.

Table (8) Relation between the Total QOL of the Studied Thalassemic Children and Compliance with Chelation Therapy

QOL scale	Compliance with chelation therapy				X^2	P-value
	Yes n = 41		No n = 59			
	No.	%	No.	%		
Child's report:						
Good	22	53.7	15	25.4	8.5	.014*
Fair	18	43.9	40	67.8		
Bad	1	2.4	4	6.8		
Parent's report:						
Good	15	36.6	6	10.2	10.5	.005*
Fair	22	53.7	42	71.2		
Bad	4	9.8	11	18.6		

According to child's report, 53.7% of compliant children had good QOL compared to 25.4% of children who were not compliant. Compliant children who had fair score constituted 43.9% compared to 67.8% of not compliant children, while 2.4% of compliant children had bad QOL compared to 6.8% of non-compliant children

According to parent's report, 36.6% of compliant children had good QOL compared to 10.2% of not compliant children. About 53.7% of compliant children had fair score compared to 71.2% of not compliant children. On contrary, 9.8% of compliant children had bad QOL compared to 18.6% of not compliant children

In addition, this table also reveals that there was statistical significance between the total QOL of the studied thalassemic children and compliance with chelation therapy in both child and parent's report (P value is statistically significant at < 0.05).

4. Discussion:

The present study showed that more than half of the studied children were males. This finding is in consistent with the findings of Elsaid (2009) who conducted his study at Zagazig university Hospitals, Egypt and Salama et al (2006) who did his study at Mansoura University Children's Hospital, Egypt. The present study is also supported by Shaligram et al (2007) and Akbar (2004) who done his study in Shiraz city.

Similar to Shaligram et al (2007), the present study showed that more than three quarters (77%) of children were from rural area compared to 23% were from urban area. The findings of the present study are in contrast with Gharaibeh et al (2009) who did his study at the National Thalassemic Center in Damascus, Syria. Gharaibeh's study illustrated that about three quadrants were from urban. This reflects that thalassemia may be present in urban as in rural when premarital screening and counseling is neglected.

The current study found that most families reported insufficient family income. This finding goes in line with the findings of Gharaibeh et al (2009). This may explain that only poor families are more likely to take their children to general hospitals, or may reflect that rich families have enough money to make premarital and prenatal screening tests when they are knowledgeable about it.

Akbar (2004) noticed that about half of cases were outcomes of first-or second-cousin marriages. This goes in line with the present study, which revealed that more than half of parents of the studied children were relatives. This may be due to strong family relationships in Egypt, especially those

who live in rural areas. In addition, educational programs about genetic counseling are still neglected.

Gharaibeh et al (2009) found that more than two thirds of children (65.3%) had sick relatives with thalassemia. This goes in line with the present study. This may be as result of increased consanguineous marriages.

The present study found that more than half of children had no compliance with iron-chelation therapy. This finding is supported by Elsaid (2009) who done his study at Zagazig university Hospitals, and he found that most of his studied sample were not compliant with iron-chelation therapy. This may be due to the painful insertion of the needle subcutaneously, long periods of infusion or limited activity during the use of desferal bump. In addition to side effects of oral chelators which include abdominal pain, diarrhea and vomiting.

Regarding the total QOL of children with thalassemia major, Shaligram et al (2007) found that three quadrants of the studied thalassemic children had poor QOL. This goes in line with the present study as more than half of children had fair QOL and 5% had bad QOL.

The present study noticed that mothers related QOL of their children as fair in more than two thirds of them and bad in 15%. This finding was supported by Shaligram et al (2007) who mentioned that caregivers related QOL of their children as poor in most children. This was due to sense of guilt toward those children as thalassemia is an inherited disease and due to the permanent comparison between them and the other healthy children or siblings .

The current study found that emotional functioning scored the lowest followed by physical then school and social functioning. This finding is in consistent with Cheuk's et al (2008) study that conducted in Hong Kong. On other hand our finding is in contrast with both Thavorncharoensap et al (2010) and Ismail et al (2006) who found that school functioning scored the lowest, followed by emotional functioning and social functioning then physical functioning. Ismail and Thavorncharoensap et al (2010) clarified their finding by the fact that frequent absenteeism from school for hospital visits, and lack of energy when performing academic activities, had a significant negative impact on the children's health related quality of life (HRQOL).

Regarding physical QOL, Shaligram et al (2007) found that the majority of children had no problems with self-care followed by usual activities, and mobility. This finding is in agreement with the present study as 79% had no problems with self care, and more than two thirds had no problems with walking. Dahlui et al (2009) clarified that, the physical function scores were higher than the other domains because these patients had been having the disease since childhood, they were not working for a living and as such had not much expectation with regard to physical performance.

The present study found that slightly less than half had bad physical QOL regarding running while, one quadrant had bad physical QOL regard making activities and exercises. These findings are supported by Caro (2002) who found, less than one quadrant of conventionally treated thalassemia major patients had their activities very often stopped due to thalassemia, its complications or desferrioxamine treatment, and 20% had their physical activities limited at least a bit. This may be due to the regular period of mild anemia before the scheduled transfusion which might limit their exercise capacity as thalassemia leads to low hemoglobin level resulting in fatigue and general weakness (Cheuk et al, 2008).

The present study found that nearly half of children had affected QOL regarding pain and less than one quadrant had bad and very bad QOL. This finding is supported by Shaligram et al (2007) who clarified his result on the basis that iron chelating therapy produce arthritis, abdominal pain, diarrhea and vomiting which may have a bearing on the high score on the pain. In addition, Telfar et al (2005) mentioned that the fully-chelated patients had a QOL almost similar to that of normal children except with regard to body pain as he clarified that was because of complications and side effects of thalassemia treatment.

Regarding emotional QOL, the present study found that more than one third of children had fair emotional QOL and only 21% had bad emotional QOL. These findings are in agreement with a subsequent multi-center European study conducted by Sadowski et al (2002) found the same results. In addition, Pradhan et al (2003) found that more than two thirds of children had emotional problems.

Emotional QOL was affected because thalassemic children feel different from their peers and elaborate negative thoughts about their life. Children at this age are becoming more aware of themselves as individuals." They work hard at "being responsible, being good and doing it right." They are now more reasonable to share and cooperate but the disease prevents them from being industry and the sense of inferiority may develop instead (Thavorncharoensap M, et al.2010).

In addition, children may develop psychological and emotional problems early from the toddler stage. Toddlers want to become capable of satisfying some of their own needs to develop a sense of autonomy but, if caregivers refuse to let them

perform these tasks due to their illness, they may develop shame and doubt about their ability to handle problems. Moreover, the treatment is emotionally demanding, as transfusion and chelation therapy require repeated invasive procedures and hospital visits.

Regarding social functioning, Gharaibeh et al (2009) reported that stigmatization was significantly noticed among older children to younger children. He also found that all children who were not currently at school experienced severe stigmatization due to thalassemia major. While among those who were currently at school, 10.2% experienced a severe level of stigmatization. This explores that the young children who were at age of 6-12 years didn't feel disease stigma so, the majority of those children had very good level of social interaction with peers and school friends.

In consistent with Gharaibeh findings, the present study found that 79% of children, who had no teasing from others, had very good social QOL. Only 10% of children, who had teasing from others, had severe problems with social interaction.

In addition, the majority of children had no problems with getting along with other kids and they related their social QOL as very good. Regarding being a member in a play team with other kids, about three quadrants of the studied thalassemic children had no problems as other kids didn't refuse them so; they related their social QOL as very good. This may be due to the nearly absence of the disease complications at this age resulting in decreased feeling of stigmatization that causes limitations of social interaction. Also, (Musallam et al, 2008) mentioned that having an adjusted family has great benefits for thalassemic children as this enhances the child confidence and ability of being socially accepted.

Gharaibeh et al (2009) clarified that the difficulty of social interactions was reported less among children with thalassemia and this might be related to strong family relationships in Arabic culture.

Regarding school functioning, Saeed (2004) explored that having to go to hospital for blood transfusion and missing school is one of the most important factors affecting the QOL of conventionally treated thalassemic patients. In addition, Gharaibeh et al (2009) reported that education was one of the greatest difficulties that affected children with thalassemia, as 42.7% of children with thalassemia experienced moderate to severe difficulties in their education. Similarly, Cantaan et al. (2003) mentioned that education of two thirds of children with thalassemia at school age was affected, mainly due to having attended hospital for investigations and transfusions. Consistent with the previous studies, the present study found that slightly less than half had fair school QOL while, only 17% were bad.

Thavorncharoensap et al (2010) found no significant relationship between frequency of blood transfusion per year and health related quality of life (HRQOL). This finding is in agreement with the current study. Schrier (2004) clarified that regular transfusions should be started when the hemoglobin concentration falls below 7 gm/dL or when there is impaired growth because those patients who are able to maintain a hemoglobin concentration more than 7.5 gm/dL usually don't require chronic transfusion therapy. This may reflect that frequency of blood transfusion per month is associated with the pre-transfusion hemoglobin level and the severity of the disease.

Salama et al (2006) discussed that life expectancy of patients with thalassemia has greatly improved over the last decade because of regular transfusions and increased compliance with iron chelation therapy. In agreement with the current study, it was found that more than half of children who were compliant with blood transfusion therapy had good QOL compared to less than one quadrant of children who were non-compliant. This may be clarified by Dubey et al. (2008) who mentioned that regular blood transfusion promotes normal growth and physical activity, suppress erythropoisess and prevent chronic hypoxia and early splenomegaly and/or hypersplenism. Therefore, it is now possible for a thalassemic child to have a near normal life span with a good quality of life.

Telfar et al (2005), discussed that QOL for non-chelated and fully chelated thalassemia patients differed. The fully chelated patients had a QOL almost similar to that of normal children. In consistency, the present study revealed that more than half of children who were compliant with iron chelation therapy had good QOL and more than one third had fair score. On other hand, one quadrant only of non-compliant children had good QOL and more than half had fair score. This may be clarified by the fact that chelation therapy can reduce complications, and improve survival and quality of life of transfused patients (*Cianciulli*, 2009).

The current study revealed that more than half of children who were not compliant with iron chelation therapy had fair QOL and the minority of both compliant and not compliant children had bad QOL. These results may indicate that non compliance with chelation therapy had minimal effect on QOL of school-age thalassemic children. From the researcher point of view, this is not a fact because children did not leave the treatment completely; they only escaped

for one or two days per week from the chelation regimen so, they had fair but not bad QOL. Only children who either completely escaped from chelation or chelation was neglected by parents had bad QOL.

5. Conclusion:

Based upon the results of the present study, it was concluded that thalassemia as a chronic disease had a negative impact on perceived physical, emotional, social and school functioning of school-age children resulting in impaired quality of life. The study reflected that emotional functioning was the most affected from the point of view of both children and parents. The study also demonstrated an association between compliance with treatment regimen and quality of life.

Based upon the finding of the current study, it may be recommended that:

- A psychologist in the hematological units and out patient clinics is a must be present to help in providing a link between patients, school officials, the families, and the physicians.
- An age-appropriate cartoon illustration for children including detailed scientific information about the disease and its survival should be provided.
- Mass media should have a role in providing information to population about Thalassemia and other inherited diseases (may be genetic counseling), and both discourage and highlight the disadvantages of consanguineous marriage.

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Saccharomyces cerevisiae and Probiotic Bacteria Potentially Inhibit Fumonisin B_1 Production in Vitro and in Vivo

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Abstract: The objective of the present study was to evaluate the efficacy of probiotic bacteria: *Lactobacillus rhamnosus* GG (LGG), *Lactobacillus rhamnosus* (LC705) and *Saccharomyces cerevisiae* (*S.cerevisiae*) to inhibit *Fusarium moniliform* (*F. moniliform*) growth *in vitro* and to eliminate fumonisin B_1 from body of mature rat *in vivo*. *S.cerevisiae*, LGG and LC 705 potentially inhibited *F. moniliform* growth and fumonisin B1 production in YES liquid media. The biologically active microorganisms (S.cerevisiae, LGG & LC705) had no toxic effects in rats when orally administered single doses of *S.cerevisiae* (10^{11} CFU ml^{-1}) and LGG & LC705 (10^9 CFU ml^{-1}). Moreover, daily treatments for 15 days with the three microorganisms in saline concomitant with FB1 in corn oil (5 mg/ml FB1), produced *by F. moniliform*, exhibited significant reduction in serum ALT, AST, GGT, creatinine, and BUN compared with the positive control group *(F. moniliform*). Blood glutathione (GSH) level significantly increased ($P< 0.05$) in groups treated with single-treatment of *S.cerevisiae*, LGG & LC705 or with fumonisin B_1 containing media. However, fumonisin B_1 - treatment severely depleted GSH level than other treatments. The best results found in *S.cerevisiae* > LGG > LC705 -YES media containing fumonisin B_1. The tested microorganisms are safely to use as food additives or preservative due to their antioxidant activity. Our study needs further continuation in this respect.
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Keywords: *Saccharomyces cerevisiae*, Probiotic bacteria, Fumonisin B1, *Fusarium moniliform*, rat, ALT, AST, GGT, creatinine, BUN and GSH.

1. Introduction

Fumonisins are a group of naturally occurring mycotoxins produced primarily by two fungi, *Fusarium verticillioides* and *F. proliferatum*, which frequently are found in corn. Fumonisins have been implicated in field cases of equine leukoencephalomalacia (Ross *et al.*, 1993), and porcine pulmonary edema (Osweiler *et al.*, 1992). Experimentally, fumonisins cause liver damage in all species studied to date, including pigs (Osweiler *et al.*, 1992), horses (Ross *et al.*, 1993), cattle (Osweiler *et al.*, 1993), sheep (Edrington *et al.*, 1995), rabbits (Gumprecht *et al.*, 1995; Ribeiro *et al.*, 2010), and rats (Suzuki *et al.*, 1995). Fumonisins also cause species-specific target-organ toxicity, such as in horses brain (Ross *et al.*, 1993), heart in pigs (Casteel *et al.*, 1994 ; Constable *et al.*, 2000; Smith *et al.*, 1999), kidney in sheep (Edrington *et al.*, 1995), rabbits (Gumprecht *et al.*, 1995; Laborde *et al.*, 1997), and rats (Direito *et al.*,2009; Suzuki *et al.*, 1995) as well as esophagus in rats and pigs (Casteel *et al.*, 1994). Epidemiologic data also has suggested an association between ingestion of contaminated corn with *F. verticillioides* and human esophageal cancer (Sydenham *et al.*, 1991). While administration of fumonisin has been fatal in a number of species, the cause of death has only been determined in pigs, where fumonisin causes acute left-sided heart failure and pulmonary edema consistent with sphingosine-mediated L-type calcium channel blockade of the heart and systemic vasculature (Constable *et al.*, 2000; Smith *et al.*, 1999 and 2000)

Probiotics are living microorganisms that when ingested may help to maintain the bacterial balance in the digestive tract of mammals, and may be included in the treatment of pathological conditions, such as diarrhoea, candidiasis, urinary infections, immune disorders, lactose intolerance, hypercholesterolemia, and food allergy (Shah, 2000; Mombelli and Gismondo,2000). They also have antigenotoxic effects; for example, species of Lactobacillus, Streptococcus, Lactococcus, and Bifidobacterium, have shown antimutagenicity in the Ames test, and their ability to decrease DNA damage in colon cells treated with N-methyl-N-nitro-N-nitrosoguanidine *in vitro* study (Pool- Zobel *et al.*,1996).

Saccharomyces cerevisiae **(***S.cerevisiae*), in particular, has proven to benefit health in several ways

including stimulation of the growth of intestinal microflore in mammals; pH modulation in ruminants (which gives rise to an increase in the rate of celulitic bacteria), improvement of reproductive parameters in milk cows and fowls (fertility and fetal development), as well as reduction in the number of pathogenic microorganisms in monogastric animals (Dawson, 1993; Wallace, 1998). In addition, a study in mouse revealed that a component of the Sc cell wall (glucan) reduced the frequency of micronuclei induced by cyclophosphamide (Chovatovicova and Mavarova, 1992).

The aim of this study is to investigate the efficacy of probiotic bacteria (LGG and LC 705) and *Saccharomyces cerevisiae* to inhibit *F. moniliform* growth *in vitro* and to eliminate fumonisin B_1 from body of mature rat *in vivo*.

2. Material and Methods

Materials:

1. HPLC using Sep-pak silica cartridge C18 columns.
2. Freeze-dried powder of *Saccharomyces crevisiae* (baker's yeast strain) and lactic acid bacteria *(Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* LC 705) were obtained from Valio Ltd. Helsinki, Finland.
3. Fumonisin and Cultures : (a) Potato dextrose agar (PDA) , (b) de`Mane- Regosa-Sharp (MRS), (c) Malt Extract Agar (MEA) and (d) Yeast extract-malt extract-sucrose broth medium were obtained from Sigma Chemical Company, P.O. box 145508, St., Lous, USA.
4. Diagnostic kits were purchased from Boehringer Mannheim GmbH Diagnostica, E.Merck, Postfach 4119, D-6100, Dramstadt, Germany. All other chemicals were of highest quality available and were obtained from commercial sources.
5. Animals: The study with Sprague Dawley rats was approved by the committee of ethics and biosecurity at NRC, Cairo, Egypt. The experiment was made using mature male rat weighing 120-130 gm b.wt., purchased from Animal House colony. Animals were divided into equal groups (six rats each) housed under standard environmental conditions (23 ± 1°C, 55 ± 5% humidity and a 12-h light: 12-h dark cycle) and maintained on a standard laboratory diet *ad libitum* with free access to water.

(1) In Vitro Study

(a) Preparation of *F.moniliform* spores and fumonisin B_1 extraction.

Cultures of *F.moniliform* were growing on potato dextrose agar (PDA) slants for 7 days at 25 °C (Bullerman,1986). The librated fumonisin B_1 were analyzed according to AOAC (2000) and quantified by HPLC technique (Sep-pak silica cartridge C18 columns) according to method's of Le Bars *et al.* (1994)

(b) Spores of Lactic acid Bacilli strains (*Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* LC705) were cultured on MRS broth / agar at 37°C until a concentration of 10^9 bacteria / ml was obtained ;counting of viable bacteria was performed by both traditional plate counting and flow cytometry (FCM) method. Bacterial counts were expressed as colony-forming units (CFU) per ml media. Viability of bacterial populations was assessed by using SYTOX ® green nucleic acid stain(Molecular probes, S-7020)at 1 µM/10^6 -10^7 bacteria to detect non viable bacteria. A band pass filter of 525 nm was used to collect the emission for green SYTOX (El-Nezami *et al.*,1998).

(c) Preparation of yeast suspensions:

The concentration of 10^{11} viable cell/ gm was determined for the probiotic yea-Sacc^{1026} through twelve decimal dilutions made in saline solution. Organisms were seeded in Petri dishes containing Sabourad broth and incubated for 72 h at 25°C and then counted for viability (Tejada de Hernandez, 1985).

(d) Inhibition experiments:

Inhibition of mold growth in the presence of *S. cerevisiae* , *Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* LC705 was performed on YES liquid media according to the method's of Bjornberg, and Schnurer (1993).

(2) In Vivo Study

Eight groups of normal rats (6 rats each) the first four groups (1, 2, 3, and 4) were orally administered *Lactobacillus rhamnosus* strain GG (LGG) , *rhamnosus* strain LC705 (LC 705) and *Saccharomyces cerevisiae (S.cerevisiae)* (10 ml / Kg b.wt.; 10^9 CFU/ 1 ml distilled water , while the control group (group 4) was given distilled water (10 ml /Kg b.wt.). The second four groups (5, 6, 7 and 8) were orally administrated fumonisin B_1 (10 mg /kg b.wt. in 10 ml corn oil) FB1 alone and /or in concomitant with LGG and LC 705, 10^{10} bacteria /ml and *S.cerevisiae* 100 µg cell/ ml water (as previously mentioned concentration). The number of rats in group 5 was 10 animals (fumonisin B_1 - treated rats) to avoid missing data in case of increase the mortality rate among this group (we choose random healthy a life 6 rats for blood samples.

After 15 days of the daily treatment; all animals were sacrificed and blood samples were collected from retro-orbital venus plexus in two test tubes : the first was heparinized test tube (imbedded in ice box) for determination of reduced glutathione (GSH) in whole blood by Ellman's method (1959) , the second test tube was plain for serum separation (1500 rpm for 15 min) to assess the following biochemical analysis: γ-glutamyl transferase (GGT) according to Rosalki *et. al*. (1970), aspartate and alanine aminotransferase (AST and ALT) activities and creatinine according to

the method of Thefeld *et al.*(1974), and BUN (Henry *et al.*,1974) using BioMerieu kits.

Statistical analysis: The obtained results analyzed by ANOVA (one or two-way) using Excel 2003 Microsoft Corp (11.5612.5606), Redmond, WA software package.

3. Results

Saccharomyces cerevisiae greatly reduced *F. moniliform* growth in YES media at concentration of 10^6 CFU/g from the first week of incubation. Meanwhile, probiotic bacteria did not alter *F. moniliform* -growth at the 2st week of incubation. Presence of FB1 in YES media resulted weak inhibitory effect.

It was clearly demonstrated that the addition of the tested biocontrol microorganisms to YES media containing *F. moniliform* all of them significantly inhibited FB1 production and mycelium growth in a variable degrees of inhibition. Fig.(1) showed that the production of fumonisin B_1 was inhibited by all studied biocontrol microorganisms, particularly, LGG was the most potent inhibitor for fumonisin B_1 production than *S.cerevisiae* or L705. The percentage of inhibition of fumonisin B_1 production was 92.88%, 89.00% and 78.64% for LGG, *S.cerevisiae* and LC 705 respectively, when compared with their respective controls (Fig. 1).

Dry weight of *F.moiliform* –mycelium also decreased significantly in YES media containing S.cerevisiae, LB GG or LB 705, the effect was 86.46%, 89.13% and 71.80%, respectively (Fig. 2).

It was found that *S.cerevisiae* and LGG were closely similar in their effect on fumonisin B_1 production and mycelium growth; in which they possess most effective agents than the effect of L705 in *F.moiliform* -YES media.

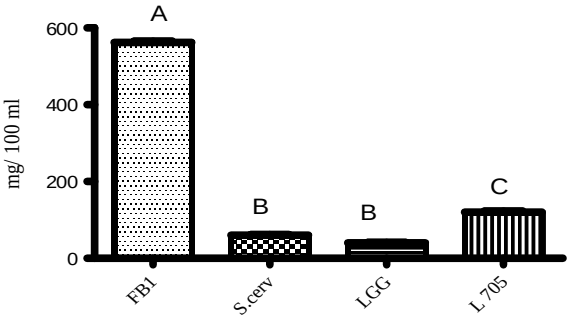

Fig. 1: Effect of S.cerv, LGGand L705 on FB1 production by *F. moniliform* in YES medium

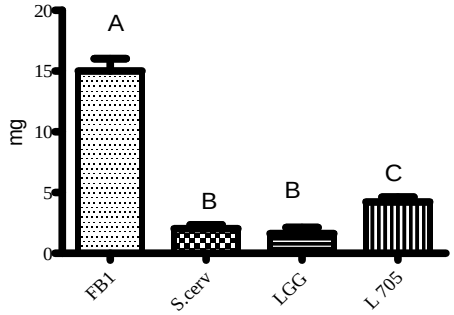

Fig. 2: Effect of S.cerv, LGG and L705 on *F. moniliform*- mycelium growth dry weight in YES medium

In vivo results:

Biochemical results (Table 1) showed the effect of different treatments on the liver enzymes (ALT, AST and GGT), kidney function tests (BUN and creatinine) and on GSH level in blood of all groups.

Single treatment with probiotic bacteria (LGG and LC 705) showed non-significant changes in ALT, AST and GGT activities comparing with the control group. While, these enzymes significantly inhibited by the treatment with *S.cerevisiae* alone. BUN values did not altered than control values in groups treated with *S.cerevisiae* or with the two strains of probiotic bacteria. Whereas, there were significant decrease in creatinine and significant increase in GSH levels in these single treated groups when compared with the controls or the other treated groups (Table 1). Significantly elevation in amino-transferases activities in the group administered FB_1 alone; this elevation was normalized by co-administration with LGG, while the combined treatment with *S. cerevisiae* and LC705 meliorated the increased activities of transaminases toward the normal figures.

FB1-administration also affected the kidney functions; BUN and creatinine values elevated significantly ($P < 0.05$) than other treatments (Table 1). Combined treatments with *S.cerevisiae*, LGG and L705 concomitant with FB_1 resulted significant decrease ($P < 0.05$) in BUN values than FB_1-treatment alone but still increased than normal groups. Creatinine had similar results as obtained in BUN values, in which the combined treatments of probiotic bacteria (LGG & LC705) with FB_1 resulted a significant decrease in creatinine level than FB_1 alone; except the group treated with S.cerevisiae plus FB_1, its creatinine value was normalized showed non-significant changes than control group.

GSH level was depleted in rat administered FB_1 alone, and it was increased significantly by the combination with LGG, LC 705 and *S.cerevisiae* to become more than normal control values.

Table (1): Effect of different biologically active microorganisms on fumonisin B1 (FB1) in rats administered 10 mg/Kg.b.wt./PO of media containing FB1 alone and /or *Saccharomyces cerevisiae* (*S.cerevisiae*) , *Lactobacillus rhamnosus* GG (LGG) and *Lactobacillus rhamnosus* LC 705 (LC 705). (n= 6 rats / group, means ± SE of the means).

Parameters	Normal groups				FB1- treated groups			
	Control	LGG	LC 705	*S. cerevisiae*	FB1	F B1+ LGG	FB1+ LC 705	FB1+ *S.cerevisiae*
ALT IU/ml	36.64± 0.86 ^{A}	35.73 ± 0.81 ^{A}	34.55± 0.77 ^{AB}	32.67± 0.68 ^{B}	66.17± 1.48 ^{C}	39.27± 0.65 ^{D}	45.22± 0.98 ^{E}	42.15± 0.73 ^{F}
AST IU/ml	44.19 ± 0.65^{AD}	42.63± 1.32 ^{AB}	41.45 ± 0.87 ^{AB}	40.47 ± 1.12 ^{B}	71.56 ± 2.39^{c}	51.38 ± 1.27 ^{D}	49.82 ± 1.33 ^{D}	47.88 ± 1.57 ^{D}
GGT IU/ml	0.97 ± 0.018 ^{A}	0.93± 0.04 ^{A}	0.82 ± 0.023 ^{A}	0.85 ± 0.029 ^{A}	3.21 ± 0.16 ^{B}	1.52 ± 0.031 ^{C}	1.56 ± 0.040 ^{C}	1.28 ± 0.030 ^{D}
GSH mg/ dl	37.33 ± 0.78 ^{A}	40.67 ± 1.3^{BE}	42.43 ± 0.97 ^{BC}	43.67 ± 0.86 ^{C}	25.18 ± 0.73 ^{D}	39.73 ± 0.75 ^{E}	41.02± 0.87 ^{BE}	43.12 ± 0.81 ^{BC}
BUN mg/ dl	23.55 ± 1.04 ^{A}	22.47 ± 0.75 ^{A}	20.98 ± 0.61 ^{A}	21.52 ± 0.89 ^{A}	35.00 ± 1.10 ^{B}	29.28 ± 1.12 ^{C}	27.23 ± 0.47 ^{C}	28.52 ± 1.26 ^{C}
Creatinine mg/ dl	0.539± 0.025 ^{A}	0.525 ± 0.071 ^{B}	0.493 ± 0.019 ^{B}	0.485 ± 0.016 ^{B}	1.075 ± 0.091 ^{C}	0.729 ± 0.030 ^{D}	0.654 ± 0.042 ^{D}	0.518± 0.017 ^{A}

ANOVA – one way

The different capital letters are significantly different at P< 0.05

4. Discussion

During the last decades, several studies have suggested that *Lactobacillus rhamnosus strain GG (LBGG)* and *L. rhamnosus strain LC-705 (*LB705) and yeast (*Sacharomyces cerevisiae*) are noted for their ability to bind mutagens *in vitro* studies (Hosono *et al*.,1990 and Yiannikouris *et al*., 2004).

In vitro study has shown that probiotic bacteria significantly reduced spores germination of *F.moniliform* and *Aspegillus flavus* (Smith,1978 and Nada *et al.*, 2010). Subsequently, they reduced mold growth and mycelial dry weight. These findings may due to the presence of aromatic compounds, such as organic acids, esters, alcohols, aldehyded, lactones and terpenes that produced by *Sacharomyces cerevisiae* (Janssens *et al.*,1992); and several antimicrobial compounds produced by probiotic bacteria which reduce fermentation products: lactic, acetic formic, propionic acids and hydrogen peroxide (Lindgren and Dobrogosz 1990).

In vivo study, the oxidative stress induced by aflatoxicosis administration was clearly observed as a model of food carcinogen (Abdel-Wahhab *et al.*, 1998 and Zhou *et al.*, 2001). Where mycotoxin (FB_1) toxicity appeared as a significant alteration in biochemical parameters and 20% deaths occurred among FB_1-treated group during the experimental period.

The explanation of this effect could be FB_1 biotransformation gives rise to various metabolites, it may covalently binds to DNA and to protein, which then alters enzymatic processes, such as glyconeogenesis, kreb's cycle, or fatty acid synthesis (Lesson *et al.*, 1995). Co-administration of FB1 with LGG, LC705, and *S.cerevisiae* diminished this oxidative damage.

The positive results are congruent with a study made in mice with the same probiotic for one week in concomitant with Ochratoxin A (OA) (Farag *et al.* 2010), where significant improvement were detected in weight level, probiotics counteracting the oxidative stress, prevent genotoxicity and spermatotoxic alterations induced by OA.

Madrigal-Santillan *et al.*(2006) studied the effect of *S.cerevisiae* (1x 10^8 live cells / g, 0.3% conc.) in mice fed FB_1 contaminated corn (0.4 and .08 mg /Kg) for 6 weeks. Authors found that *S.cerevisiae* improved the loss of weight gain and it had a potent adsorbent capacity without structural modification in FB_1 molecule. Recently, Nada *et al.*(2010) investigated that *S.cerevisiae* and probiotic lactic acid bacilli had protective effect against aflatoxicosis in rat when they orally administrated with aflatoxin B_1 for 15 days and ameliorated liver and kidney tissues from AFB_1toxicity.

Few literatures were dealed with the isolated cell wall of *S.cerevisiae in vitro* study, and proved the

presence of B-D-glucans in adequate percentage on the cell wall of *S.cerevisiae* spp. consequently, it has antioxidant activities, (Yiannikouris *et al.* 2003); Oliveira *et al.* (2009) and Sener *et al.* (2007) found that B-glucans reduced DNA-oxidative damage through scavenging of both •OH radicals and singlet oxygen species *in vitro* study.

Live *Sacharomyces cerevisiae* has been used as food supplement (due to its high content of vitamins, particularly those of the vitamin B group, minerals, proteins and enzymes), and it is employed as a biotherapeutic or probiotic agent for re-equilibration of the intestinal flora. Some clinical studies have shown that these preparations are effective for the treatment of chronic diarrhea, especially those cases associated with parenteral nutrition and/ or super infection with *Clostridium difficile* (Bleichner *et al.*, 1997; Guslandi *et al.*, 2000). *Sacharomyces cerevisiae* therapeutic effects is attributed to its release of a 54-kDa protease that causes cleavage of *C. difficile* toxins A and B and diminishes their capacity for binding to receptors located on the human colonic brush-border membrane (Castagliuolo *et al.*, 1999) , this hypothesis may support our results in preventing fumonisins in the current experimental study.

Reduced glutathione (GSH) is the main component of endogenous non-protein sulfhydryl pool that scavenges free radicals in the cytoplasm (Ross 1988, Shaw *et al.*,1990). Because of their exposed sulfhydryl groups, non-protein sulfhydryls bind a variety of electrophilic radicals and metabolites that may be damaging to cells (Szabo *et al.*, 1992). In fact, antioxidants maintain the concentration of reduced GSH, which may restore the cellular defense mechanisms, block lipid peroxidation, and thus protect against the oxidative tissue damage (Toklu *et al.*, 2006).

B-Glucans are glucose polymers found in the cell walls of yeast, fungi, and cereal plants. The beneficial effects on the immune system and the lack of toxic or adverse effects (Vetvicka, 2001 and Sener *et al.*, 2007), had focused the studies on B-glucan molecule.

Currently, B -glucans are accepted to be one of the most powerful immune response modifiers (Brown and Gordon, 2003); it inhibits tumor development, enhances defense against bacterial, viral, fungal, parasitic challenge (Onderdonk *et al.*,1992; Kernoddle *et al.*, 1998). B–glucans activates macrophages (Cleary *et al.*, 1999; Vetvicka and Yvin, 2004). It induces production of cytokines (Soltys and Quinn, 1999; Engstad *et al.*, 2002); nitric oxide (NO), arachidonic acid metabolites (Ljungman *et al.*, 1998) and increases hematopioesis. As well as, B-glucans exerts radio-protective effects, improves wound healing by inducing the macrophage release of wound growth factors (Wei *et al.*, 2002) and lower serum lipids (Nicolosi *et al.*, 1999). Several mechanisms were proposed for the protective effect of B-glucan, one of them is related to antioxidant capacity of the molecule (Babincova *et al.*, 2002; Krizkova *et al.*, 2003; Sener *et al.*, 2005). Yiannikouris *et al.*, (2003 and 2004) and Young *et al.*,(2000) studied the adsorption capacity of yeast cell wall and the role of various B-D-glucan types in the efficacy of Zearalenone adsorption and thought to elucidate some of adsorption mechanisms *in vitro* studies with Zearalenone .

The potential function of these specific strains may be due to the capacity to reduce the carcinogenic or toxic effect of food carcinogens by binding to them or metabolically transforming them into toxic carcinogenic degradation products (Zhou *et al.*, 2001).

Our results indicated such effect by selected strains of *S.cerevisiae* and their ability to remove FB_1 from contaminated culture media. *S.cerevisiae* could greatly prevent FB_1 toxicity and improve liver and kidney markers; enhance GSH synthesis. *S.cerevisiae* is noted for its ability to bind mutagens (Hosono *et al.*, 1990). El-Nezami *et al.*, (1998) found that the maximum of $2X10^9$ CFU/ml was required for significant of aflatoxins removal (99%) by LGG and LC705 in vitro study.

Similar results in another strain (*Bifidobacteria*) were reported by Oatley *et al.* (2000) , where authors found *Bifidobacteria* bound to 25% - 60% of the FB1 added to the media.

Our study was in agreement with hypothesis that *S.cerevisiae* and lactic acid bacilli colonies could to diminished FB_1 (a model of food carcinogens) and protect experimental animals from its oxidative stress (Nada *et al.*, 2010) , in which *S. cerevisiae* administration concomitant with FB_1 gives us the best results than that with probiotic bacteria .

Conclusion, our results improved that, the tested probiotic bacteria and yeast had no toxic effects with their free radical scavenging and antioxidant properties, seems to be a highly promising agent in preventing food contamination with *F.moniliform* and greatly inhibit toxin production *in vitro* as well as they succeeded to protect various tissues against FB1-induced oxidative damage .

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Correlation between Caregivers' Burnout and Elderly Psychological Abuse

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Abstract: Psychological abuse of elders is a growing but hidden problem and is often under reported. Aim: this study aims to investigate the correlation between caregivers' burnout and elderly psychological abuse. Design: A descriptive correlational research design was utilized to conduct this study. Sample :It included 150 older person residing Dar El-Deiafaa, Dar El-Salam and Dar El-Zahraa for disabled and elderly people and 50 of caregivers (nurses& elderly sitters) ,who are working in these settings. Tools of data collection: include,1) socio-demographic data sheet concerned with caregivers' personal characteristics,2) Burnout Inventory developed by Maslach (1981),it was modified and translated into Arabic by the researchers and 3) Elder Abuse Screening Instrument developed by Fulmer et al (2004), that was modified and translated into Arabic by the researchers. Results: the study results revealed that, 34% of the studied caregivers their ages ranged from 35 to 40 years, 62% were male,52% their education at secondary stage & only 8% had university degree. Majority of them 64% worked as elderly sitter and 36% were nurses. 62% were unsatisfied with their paid, and 38% were satisfied with their paid. 58% had experience less than 5 years in their working with the elders, but 6% only had experience more than 10 years. Conclusion: There are strong positive associations between levels of caregivers' burnout and levels of elders' psychological abuse. Recommendations: I t is recommended that media coverage of abuse in elders homes has made the public knowledgeable about-and outraged against-abusive treatment in those settings, providing education, appropriate training and counseling for the caregivers to find solutions for their problems and the problems of the elderly and about the risk factors for abuse.

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Key words: Elders, caregivers, burnout, psychological abuse

1. Introduction:

Aging is a continuous process from birth to death, which encompasses physical, social, psychological, and spiritual changes.

Although aging is an ongoing process, the value of aging is seen differently at different points in the process. Some of the changes are

anticipated with joy, other changes are greeted with a less positive response, such as gray hairs that appear (Hunt, 2004).

In fact, world wide population of individuals older than 65 years will surpass 1 billion people in 2030. This trend reflects a drastic increase in the global population. Even in many third world countries the life expectancy has increased to 80 years. By 2040, the elderly will comprise 20% of the world's population. The estimated number of individuals 80 years of age and older, will double or triple (Rutty, 2008).

In Egypt, people of age 65 and above are 4.1%of all population. Average of life span for males is 68 years, while it was 72 years for females (EDHS, 2005). Therefore, the ever expanding growth of the geriatric population increases the likelihood of elder abuse and neglect to occur, both in public and in private setting (Rutty, 2008).

Abuse shall mean an act or omission which results in harm or threatened harm to the health or welfare of an elderly person. Abuse includes intentional infliction of physical or mental injury; sexual, or withholding of necessary food, clothing, and medical care to meet the physical and mental needs of an elderly by one having the care, custody or responsibility of an elderly person. Elder abuses that may take many forms are often classified as physical abuse and neglect, psychologic abuse, financial exploitation and violation of rights (Kurrle et al., 1997).

Emotional or psychological abuse is a willful act executed to cause emotional pain, injury, or mental anguish (Kruger et al., 1999). Psychological or emotional abuse is also defined as the intentional or reckless infliction of psychological pain, injury, suffering, or distress through verbal or nonverbal acts (Carney et al., 2003). However, the victims of emotional and verbal abuse may be threatened, humiliated or kept isolated from family and friends. Moreover, the elder's self-esteem is damaged and

added that emotional abuse which is difficult to detect (Tennstedt, 1999).

However, older age, social isolation, functional debility, psychologic disorder or character pathology, cognitive impairment as well as caregiver burnout and frustration are the main risk factors for elderly mistreatment or abuse (Kurrle et al., 1997). Classic symptoms of elder emotional abuse are identified as agitation, anger, negative attitude, fearful behavior, especially around certain individuals as well an elder's report of verbal abuse or mistreatment (Carney et al., 2003).

Moreover, not all elder abuse can be seen with naked eye. There are also emotional and psychological abuses that occur when a person is demeaning and dehumanizing to another person. Psychological and emotional abuse can also make someone withdraw into depression or even deny that anything bad is actually taking place (Wilson, 2008).

Caregiver refers to anyone who routinely helps others who are limited by chronic conditions. "Formal caregivers" are volunteers or paid employees connected to the social service or health care systems (Tennstedt, 1999).

Caregiver burnout is a state of physical, emotional, and mental exhaustion that may be accompanied by a change in attitude from positive and caring to negative and unconcerned. Burnout can occur when caregivers don't get the help they need, or if they try to do more than they are able either physically or financially. Caregivers often are so busy caring for others that they tend to neglect their own emotional, physical, and spiritual health. The demands on a caregiver's body, mind, and emotions can easily seem overwhelming, leading to fatigue and hopelessness and, ultimately burnout (Montgomery &Kosloski, 2000).

In this regard, factors that can lead to caregiver burnout include role confusion, lack of control (i.e., many caregivers become frustrated by a lack of money, resources, and skills to effectively plan, manage, and organize their one's care); unrealistic expectations (i.e., many caregivers expect their involvement to have a positive effect on the health and happiness of the patient); and unreasonable demands. Furthermore, many caregivers cannot recognize when they are suffering burnout and eventually get to the point where they cannot function effectively. They may even become sick themselves (Grunfeld, 2004).

The symptoms of caregiver burnout are similar to the symptoms of stress and depression. They include withdrawal from friends, family and other loved ones, loss of interest in activities previously enjoyed, feeling blue, irritable, hopeless, and helpless, changes in appetite, weight, or both, changes in sleep patterns, getting sick more often, feelings of wanting to hurt yourself or the person for whom you are caring, emotional and physical exhaustion and irritability (Zarit, 2006).

Within institutions, elderly residents may be powerless and vulnerable and staff may be underpaid, under qualified, overworked and burned out. These factors create a climate which can contribute to elder abuse (McCreadie et al., 2000). Moreover, health care professionals also see themselves as abused by the system; especially working with older persons is considered "second class", with low wages and less qualified staff than in other areas (World Health Organization, 2002).

Therefore, health care professionals must take the lead in recognizing, reporting, and seeking help for the frail elderly. With a good reporting system in place, nurses also can follow a clearly delineated safety plan to get immediate help from social services, an elder abuse team, or administration while patient is still in the hospital or agency. Nurses must also help patients avoid feelings of embarrassment, shame, and helplessness. In addition, all nurses must be aware of adult protective services available in their communities (Cole, 2002)

Significance of the Study:

Elder abuse is a violation of human rights. Elder abuse has devastating consequences for older persons such as poor quality of life, psychological distress, and loss of property and security. It is also associated with increased mortality and morbidity (Lachs et al., 1998; Perel-Levin, 2005). Elder abuse is a problem that manifests itself in both rich and poor countries and at all levels of society (World Health Organization, 2002).

Because of differing definitions, poor detection and underreporting, the extent of elder mistreatment is unknown and firstly gained attention as medical and social problems about 20 years ago (Swagerty et al., 1999).

In this regard, institutionalized elderly who are frail and dependent are vulnerable to be abused by overwhelmed caregivers especially caregiver psychological abusive behavior. Therefore, the purpose of the study is to investigate the correlation between caregivers' burnout and elderly emotional abuse.

2. Subjects and Methods:

The aim of this study was to investigate the correlation between caregivers' burnout and elderly emotional abuse.

Research question

Is the caregivers' burnout leading to an elderly emotional abuse?

Technical design:

A descriptive correlational research design was utilized to conduct this study.

Setting:

The study was conducted at the Dar El-Deiafaa, Dar El-Salam and Dar El-Zahraa for disabled and elderly people, Amman, Jordan.

Inclusive criteria for elderly residents:
- Age: 60 years and above.
- Geriatric home residents.
-Capable of verbal communication
- Totally and partially dependent on the caregiver.\

Inclusive criteria for caregivers:
- Less qualified (without special geriatric training).
- Poorly paid
- Over worked.

Sample:

A purposeful sample of 150 older persons who reside Dar El-Deiafaa, Dar El-Salam and Dar El-Zahraa for disabled and elderly people and are matching with the determined inclusive criteria were included in the study sample. A 50 caregivers (nurses & elderly sitters), who are working in Dar El-Deiafaa, Dar El-Salam and Dar El-Zahraa for disabled and elderly people were also involved in the study sample.

Data Collection Tools

1- A socio-demographic data sheet**,** concerned with caregivers' personal characteristics was developed by the researchers. It included caregivers' age, sex, marital status, number of children, educational level, occupation, monthly income, and years of experience.

2- Maslach Burnout Inventory developed by Maslach &Jackson (1981) modified and translated into Arabic by the researchers. Maslach Burnout Inventory comprised two aspects of burnout syndrome: (a) 8 items concerned with feelings of being emotionally overextended and exhausted by one's work; (b) 10 items concerned with feelings of depersonalization /frustration (i.e., impersonal response towards recipient of one's service, care, treatment, or instruction).

Scoring system:

Every participant of the caregivers was asked to point a response with rare, frequently, always according to the frequency of the experienced feeling.

3-Elder Abuse Screening Instrument developed by Fulmer et al. (2004), it was modified and translated into Arabic by the researchers. It comprised two main aspects; 12 items concerned with elder safety and 8 items concerned with elder access of health services.

Scoring system:

Every participant of the elder residents was asked to indicate a response with rare, frequently, always according to the frequency of the exposure to these caregivers' abusive behaviors.

Operational Design:

Pilot study:

A pilot study was undertaken with the objectives of testing the tools and ensure their practicability, and to determine the clarity of the statements. It also helped to clarify the way the study will be conducted. It was carried out on 15 elder residents and 5 caregivers representing 10% of the total participants of both samples involved in the actual study.

Field work:

Once permission was granted to proceed in this study, the researchers contacted the participants (elder residents & caregivers) in the study to explain simply the purpose and the nature of the study in the light of their grasping. They were assured anonymity of answers and that the information will be used for scientific research only and will be strictly confidential. Data of this study were collected throughout three months from September to November 2008 in Dar El-Deiafaa, Dar El-Salam and Dar El-Zahraa homes for disabled and elderly people. Concerning socio-demographic data sheet and Maslach Burnout Inventory, they were distributed to the nurses and elderly sitters at their working place to fill in. Then they returned the filled in sheets to the study researchers. Meanwhile, the elder residents were interviewed for approximately 20 minutes to fill in the Elder Abuse Screening Instrument.

Statistical Design:

In order to achieve the study objective and respond to its question many of statistical procedures have been used such as frequency tables, means, and standard deviation in addition to r-test.

3. Results:

Table (1) shows that 34% of the studied caregivers their ages ranged from 35 to 40 years, while only 16% of them were under 25 years. More than three fifths of them (62%) were males. 64% of them were married and only 6% were separated .Concerning number of children the highest percentage (48%) had no children ,14% had one child, another 14% had three and 20% of them had two children. Considering education 52% of the studied caregivers, their education was at secondary stage, while only 8% had university degree. Less than two third of them (64%) work as elderly sitter while 36% were nurses. As for

monthly income 62% were unsatisfied with their pay while the remaining percentage (38%) was satisfied with their pay. Almost three fifths of the studied caregivers (58%) had experience less than 5 years in their working with the elders but 6% only had experience more than 10 years.

As regards levels of emotional exhaustion pertinent to burnout and experienced by the studied caregivers, table (2) demonstrates that the highest percentage of them representing 62%, 66%, and 68% always " feel emotionally drained from their work", "feel used up at the end of the work day "and "feel fatigued when they get up in the morning and have to face another day on the job; compared to 2%, 2%, 0% who rarely had these feelings. Regarding to levels of depersonalization/frustration pertinent to burnout and experienced by the studied caregivers, the highest percentage of them 58%, 64%, and 56% frequently " worry that this job is hardening them emotionally"," don't really care what happens to some recipients" and " became not caring with many things that used to please them previously, respectively versus 4%, 20%, 22% rarely had these feelings.

Investigating levels of elder safety pertinent to psychological abuse experienced by the studied elders, table (3) demonstrates 72.7%, 84.7% of them were always "exposed to isolation from friends or regular activities" and" "any one caused blame, harassment , or improper call naming" for them respectively. Otherwise, 3.3% only rarely " feel unsafe where they are living" compared to 62% always have this feeling.

Table (1): Distribution of the Studied Caregivers according to their Demographic Characteristics (n=50).

Item	No	%
Age in (years):		
<25	8	16.0
25-	15	30.0
30-	10	20.0
35-40	17	34.0
Gender:		
Male	31	62.0
Female	19	.0 38
Marital Status:		
Single	15	30.0
Married	32	64 .0
Separated	3	6.0
Number of Children:		
None	24	48.0
One	7	14.0
Two	10	20.0
Three	7	14.0
Four	2	4 .0
Educational Level;		
Elementary stage	20	40.0
Secondary stage	26	52.0
University degree	4	8.0
Occupation:		
Nurse	18	36.0
Elderly Sitter	32	64.0
Monthly Income:		
Satisfactory paid	19	38.0
Unsatisfactory paid	31	62.0
Years of Experience:		
<5 years	29	58.0
5-10 years	18	36.0
>10 years	3	6.0

Table (2): Distribution of the Studied Caregivers According to their Levels of Burnout (n =50).

Item	Rarely		Frequently		Always	
	No	%	No	%	No	%
Emotional Exhaustion:						
1-I feel emotionally drained from my work.	1	2.0	18	36.0	31	62.0
2-I feel used up at the end of the work day.	1	2.0	16	32.0	33	66.0
3-I feel fatigued when I get up in the morning and have to face another day on the job.	0	0.0	16	32.0	34	68.0
4-Working with people all day is really a strain for me.	0	0.0	25	50.0	25	50.0
5-I feel frustrated by my job.	0	0.0	21	42.0	29	58.0
6-I feel I'm working too hard on my job.	0	0.0	20	40.0	30	60.0
7-Working with people directly puts too much stress on me.	0	0.0	24	48.0	26	52.0
8- I feel like I'm at the end of my rope.	3	6.0	40	80.0	7	14.0
Depersonalization/Frustration:						
1-I feel I treat some recipients as if they were impersonal objects.	50	100.0	0	0.0	0	0.0
2-I've become more callous toward people since I took this job.	29	58.0	21	42.0	0	0.0
3-I worry that this job is hardening me emotionally.	2	4.0	29	58.0	19	38.0
4-I don't really care what happens to some recipients.	10	20.0	32	64.0	8	16.0
5-I feel recipients blame me for some of their problems.	22	44.0	14	28.0	14	28.0
6-I became isolated from my family, friends …..etc.	13	26.0	25	50.0	12	24.0
7-I became not caring with many things that used to please me previously.	11	22.0	28	56.0	11	22.0
8-I feel changes in my appetite &weight.	24	48.0	21	42.0	5	10.0
9-I feel changes in my sleep patterns.	24	48.0	21	42.0	5	10.0
10-I feel a desire to hurt my self or others for whom I provide care.	49	98.0	1	2.0	0	0.0

Table (3) Distribution of the Studied Elders According to their Levels of Psychological Abuse (n= 150).

Item	Rarely		Frequently		Always	
	No	%	No	%	No	%
Elder Safety:						
1-Do you feel unsafe where you are living?	5	3.3	52	34.7	93	62.0
2- Are you exposed to unauthorized physical or chemical restraint?	25	16.7	124	82.7	1	0.6
3-Are you exposed to isolation from friends or regular activities?	0	0.0	41	27.3	109	72.7
4- Has anyone caused blame, harassment or improper name calling?	0	0.0	23	15.3	127	84.7
5-Has anyone ever touched you without your consent?	11	7.3	82	54.7	57	38.0
6-Has anyone forced you to do things you didn't want to do?	4	2.7	86	57.3	60	40.0
7- Has anyone ever taken any thing or money that belongs to you without your ok?	36	24.0	102	68.0	12	8.0
8-Has anyone ever strike you with any objects.	46	30.7	98	65.3	6	4.0
9- Has any one treated you as an infant?	0	0.0	70	46.7	80	53.3
10-Has anyone withheld your consent for particular interventions?	8	5.4	50	33.3	92	61.3
11-Does anyone scold or shout at you.	0	0.0	50	33.3	100	66.7
12- Has any one tried to hurt you or harm you recently?	25	16.7	108	72.0	17	11.3
Elders Access to Health Services:						
1-Has anyone ever discriminate you from others?	0	0.0	29	19.3	121	80.7
2- Are you deprived by your caregiver from access to adequate food?	6	4.0	82	54.7	62	41.3
3- Are you deprived by your caregiver from proper personal hygiene, clothing?	6	4.0	64	42.7	80	53.3
4- Has your caregiver failed to provide necessary medical care?	39	26.0	96	64.0	15	10.0
5-Has your caregiver deprived you from proper transfers?	42	28.0	82	54.7	26	17.3
6-Does anyone make you isolated from the outer world including phone calls, visitors?	33	22.0	107	71.3	10	6.7
7- Has your caregiver failed or refused to provide services or care necessary to maintain physical or mental health?	28	18.7	88	58.6	34	22.7
8-Has your caregiver failed to provide an outlet for recreational/occupational activities.	0	0.0	39	26.0	111	74.0

Around half of the participant elders 54.7%, 57.3% are frequently" touched from someone without their consent" and " forced to do things they didn't want to do" respectively. More than half of them (53.3%) always"treated by others as infants" and 66.7% were always "scolded or shouted at them by others". Regarding to levels of elder access to health services pertinent to psychological abuse and experienced by studied elders, the highest percentage of them 80.7%, 74% always suffer from " discrimination from caregivers in their dealing with them" and " their caregivers failed to provide an outlets for recreational/occupational activities". About half (53.3%) are always also "deprived by their caregivers from proper personal hygiene, and clothing". 71.3% are frequently " isolated from the outer world including phone calls, and visitors", 64% frequently " their caregiver failed to provide necessary medical care" and 58.6% frequently "their caregivers failed or refused to provide services or care necessary to maintain physical or mental health. Compared to only 4% are rarely " deprived by their caregiver from access to adequate food" and 4% also rarely" deprived by their caregiver from proper personal hygiene, and clothing"

Table (4) shows that there are strong positive associations between levels of caregivers' burnout related to emotional exhaustion and levels of elders' psychological abuse related to feeling of safety (r=+0.97) which means that elders' feeling of safety declines with escalation of emotional exhaustion levels for either the nurses or elderly sitters as caregivers in the geriatric homes. As well, there are strong positive associations between levels of caregivers' burnout related to depersonalization/frustration and levels of elders' psychological abuse related to feeling of safety(r=+0.83) which means that elders' feeling of safety decline with escalation of depersonalization/frustration levels for the participated caregivers.

Table (5) shows that there are strong positive associations between levels of caregivers' burnout related to emotional exhaustion and levels of elders' psychological abuse related to their access of health services(r=+0.79) which means, access of health services for the participated elders declines with escalation of emotional exhaustion levels for either the nurses or elderly sitters as caregivers in the geriatric homes. Moreover, there are strong positive associations between levels of caregivers' burnout related to depersonalization/frustration and levels of elders' psychological abuse related to their access of health services(r=+0.81) which means that, access of health services for the participated elders declines with escalation of depersonalization/frustration levels for the participated caregivers.

Table (4): Correlation between Levels of Caregivers' Burnout and Levels of Elders' Psychological Abuse Related to their Feeling of Safety.

Levels of Caregivers' Burnout	Levels of Elders' Feeling of Safety			
	Rarely X±SD	Frequently X±SD	Always X±SD	r-test
Emotional Exhaustion: -Rarely	0.7±1.1	0 ± 0	0 ± 0	+0.97
- Frequently	6.3±0.9	8.7±0.8	6.3±0.9	
- Always	0 ± 0	14.3±0.4	13.7±0.4	
Depersonalization/ Frustration				
-Rarely	7.0±0.8	12.6±0.5	3.4±1.0	+0.83
- Frequently	0 ± 0	8.9±0.8	10.1±0.5	
- Always	0 ± 0	1.5±1.2	6.3±0.9	

Table (5): Correlation between Levels of Caregivers' Burnout and Levels of Elders' Psychological Abuse Related to their Access of Health Services.

Levels of Caregivers' Burnout	Levels of Elders' Access of Health Services.			
	Rarely X±SD	Frequently X±SD	Always X±SD	r-test
Emotional Exhaustion: -Rarely	0 ± 0	0 ± 0	0.7± 1.1	+0.79
- Frequently	4.3±0.8	2.3±1.6	15.4±0.4	
- Always	1.4±1.2	22.7±0.3	2.9±1.0	
Depersonalization/Frustration:				
-Rarely	4.3±0.8	13.6±0.4	7.1±0.8	+0.81
- Frequently	1.7±1.2	8.9±0.8	7.4±0.8	
- Always	0±0	2.5±1.0	4.5±0.9	

4. Discussion:

Every man, woman, and child deserves to be treated with respect and caring. Individuals of all ages deserve to be protected from harm by caregivers. Elder abuse is being recognized increasingly as a health and social phenomenon. Elder abuse is defined as the infliction of injury, unreasonable confinement, intimidation, or punishment, with resulting physical harm, pain, or mental anguish. It can also be the willful deprivation by a caregiver of goods or services that are necessary to maintain physical or mental health (American Psychological Association, 2006; Lee, 2007). Psychological or emotional abuse is a category of maltreatment. Usually defined as an act carried out with the intention of causing emotional pain or injury, psychological abuse often accompanies physical abuse (Lachs &Pillemer, 1995). The present study aimed at investigating the correlation between caregivers' burnout and elderly emotional abuse.

Regarding to levels of burnout among the studied caregivers of the resident elders, the results of present study revealed that about three fifths of them are always emotionally exhausted since they felt emotionally drained from their work and they felt used up at the end of the work day and feel fatigued when they get up in the morning and have to face another day on the job compared to nearly none of them rarely had these feelings. Additionally, around three fifths of them are frequently depersonalized/frustrated since they worry that this job is hardening them emotionally and don't really care what happens to some recipients and became not caring with many things that used to please them previously. Conversely, minorities of them rarely had these feelings. This finding could be due to that the responsibilities of caregiving to those elders who experience limitations in one or more tasks of daily living activities are the potential for stress on the individual caregivers themselves, which means that giving care to recipients with physical frailty including chronic illnesses, and physical and sensorial disabilities are leading to high levels of stress. These results are in agreement with those of the study conducted by Cocco et al. (2002), to compare levels of stress and burnout among staff caregivers in three nursing homes and nine geriatric sections of general hospitals and reached to that, the studied caregivers are experiencing higher Maslach Burnout Inventory, depersonalization and emotional exhaustion

These results are also congruent with those of the study done by Mandiracioglu et al. (2006), to describe the frequency of violence against personnel

from residents and to identify the prevalence of burnout among staff working in nursing homes. It was found that staff who had complaint about the elderly and work conditions had more exposure to violent behaviour and higher burnout scores. Within the same context, Truzzi et al. (2008) stated that emotional exhaustion is the core burnout dimension and is directly associated to high levels of work overload.

Regarding to levels of psychological abuse among the studied elders ,the results of the current study revealed that majority of them always feel unsafe because of being exposed to isolation from friends or regular activities and anyone causes blaming, harassment for them or improperly call their names. Otherwise, a minority of them rarely feel unsafe where they are living, compared to slightly more than three fifths of them always feel unsafe where they are living and two thirds are scolded or shouted at by others. Around half of them are frequently touched from someone without their consent and are forced to do things they didn't want to do. Additionally, approximately half of them are always treated by others as infants. The majority of them always lack access of health services because of their exposure to discrimination from caregivers in their dealing with them and their caregivers failed to provide an outlet for recreational/occupational activities. About half of them also are always deprived by their caregivers from proper personal hygiene and clothing. A relatively higher percentage of them are frequently isolated from the outer world including phone calls and visitors, their caregivers failed to provide necessary medical care and their caregiver failed or refused to provide services or care necessary to maintain physical or mental health. However, minority of them are rarely deprived by their caregiver from access to adequate food and from proper personal hygiene and clothing. These findings could be due to that caregiver's stress is a significant risk factor for abuse a frail, ill, partially or totally physically or mentally impaired older persons since they frequently experience intense frustration and anger that can lead to a range of abusive behaviors. In addition, certain societal attitudes make it easier for abuse to continue without detection or intervention.

The current study findings are congruent with that of the study carried out by Wang (2005) to examine the prevalence of psychological abuse and identified that individuals' characteristics were associated with different levels of psychological abuse in a group of randomly selected elderly Taiwanese. Wang clarified that psychological abuse appeared to be higher among elderly people with lower cognitive and physical functioning. This result is consistent also with the study conducted by Hsieh et al.(2008), who stated that institutionalized elderly who are frail and dependent are vulnerable to be abused by overwhelmed caregivers especially caregiver psychological abusive behaviors. Within the same context, Hawes (2002) highlighted that staff shortage and turnover are viewed as significant issues for elder abuse and neglect in residential long-term care facilities. In this regard, Post et al. (2008) pointed to caregiver's mental health and personal problems of the abuser and the personal characteristics of the elderly as primary factors of elder abuse and neglect.

The finding of this study also revealed that there were strong positive associations between levels of caregivers' burnout and levels of elders' psychological abuse. This could be due to that caregivers who are unhappy, frustrated, easily angered, and who feel entitled to lash out at others with less power may be more likely to commit some extreme forms of elder abuse. In this regard, Wang &Lee (2003) identified that female caregivers, caregivers with higher levels of burdens demonstrated more severe psychologically abusive behavior. Similarly, Lachs et al. (1998) and Compton et al. (1997) stated that elder abuse is associated with distress and increased mortality in older people and caregiver psychological morbidity. This finding is consistent with that of Wang (2005), which clarified that caregivers without special geriatric training or experiencing higher workload burdens displayed more severe psychologically abusive behaviors. Within the same context Robert and Griffith (1999) reported that "burnout" and "frustration" experienced by the caregivers can lead them to abuse their charge. Similarly, Maya and Liao (2006) confirmed that caregivers' stress and victim dependency increase the risk for abuse.

5. Conclusion:

Results of the study revealed that there are strong positive associations between levels of caregivers' burnout related to emotional exhaustion and levels of elders' psychological abuse related to feeling of safety. As well, there are strong positive associations between levels of caregivers' burnout related to depersonalization/frustration and levels of elders' psychological abuse related to feeling of safety.

Additionally, there are strong positive associations between levels of caregivers' burnout related to emotional exhaustion and levels of elders' psychological abuse related to their access to health services Moreover, there are strong positive associations between levels of caregivers' burnout related to depersonalization/frustration and levels of elders' psychological abuse related to their access of

health services. That it means, heightening in the levels of elders' psychological abuse (i.e., declining in their feeling of safety and shortage in access of health services delivered to them) are closely related to escalation of burnout levels among the participated caregivers (i.e., intense feeling of emotional exhaustion & depersonalization and frustration)

Recommendations:

In the light of the study results, it is recommended that:

By increasing awareness among physicians, mental health professionals, home health care workers, and others who provide services to the elderly and family members, patterns of abuse or neglect can be broken, and both the abused person and the abuser can receive needed help.

Appropriate training for the caregivers to be aware of the danger signs of burn-out and to have opportunities to manage their stress.

Educating the caregivers is the cornerstone for preventing elder abuse by focusing on the special needs and problems of the elderly and about the risk factors for abuse.

Media coverage of abuse in elders' homes has made the public knowledgeable about and outraged against-abusive treatment in those settings.

Counseling for the caregivers' behavioral or personal problems can play a significant role in helping them change lifelong patterns of behavior or find solutions to problems emerging from current stresses.

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Synthesis and structure-activity relationship of new cephalosporins modified at C-7 and C-4

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Abstract: The synthesis and antimicrobial activity of a series of cefaclor derivatives bearing phthalyl or tosylaminoacyl or dipeptidyl moieties attached to the α-amino group of the 7-phenylglycinamido acyl unit, or amino acid residues and their corresponding methyl esters linked to the carbonyl group on C-4 are described. Some compounds of this series were found to possess high activity against pseudomonas aeruginosa and other Gram-negative bacteria.

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Keywords: Cefaclor, amino acids, antimicrobial activity.

Introduction:

In recent years, intensive research has been carried out in order to obtain modified cephalosporins with improved antimicrobial properties[1-11]. Many derivatives of 7-acylphenylacetamidocephalosporin have been synthesized and are found to possess enhanced activity toward gram-negative micro-organisms [12-19]. As far as we know, no cephal-osporins are known in which the α-amino group of 7-phenylglycinamido acyl moiety has been reacted with phthalyl or tosylaminoacyl or dipeptidyl units. On the other hand, some cephalosporin compounds having modifications on C-4 have shown consider-able biological activity[20-22].In continuation of our work on structure-activity relationship of amino acid derivatives (*SAR*)[23-27] , these facts prompted us to investigate the introduction of a new series of cefaclor compounds containing modified acyl chains at the C-7 or amino acid amide residues at the C-4 and to determine the effect of these modifications on the antibacterial activity of cefaclor against gram-positive and gram-negative bacteria including some strains of *Pseudomonas aeruginosa* and *proteus vulgaris* which are normally insensitive to some cephalosporin antibiotics [10].

Discussion:

Cefaclor methyl ester hydrochloride derivative (II) was easily prepared by treatment of a suspension of cefaclor (I) in absolute methanol with pure thionyl chloride. The reaction mixture was stirred for 3 hours in an ice bath. The isolated ester was obtained and secured in excellent yield.

The hydrazide derivative (III) was obtained by treatment of (II) with alc. hydrazine hydrate solution. The reaction mixture was kept at room temperature for 24 hours where the hydrazide separated out. The cefaclor hydrazide was isolated, purified and obtained in high yield.

Preparation of *N*-Tos-cefaclor (IV) was performed by the action of *p*-toluenesulphonyl chloride (tosyl chloride) on cefaclor (I) in an alkaline solution. To avoid the action of strong alkaline such as sodium hydroxide on cefaclor, it was found that the most suitable alkaline medium for this preparation consists of tetrahydrofuran, water and two molar equivalents of triethylamine. After sometime, the organic layer was removed and H_2O was added and then the desired product obtained by acidification. Facial preparation of *p*-nitophenyl ester of *N*-Tos-cefaclor derivative (V) was achieved through the action of 1 equivalent of *N, N*-dicyclohexylcarbodi-imide (DCC)[28] on a solution containing 1 equivalent each of *N*-Tos-cefaclor (IV) and *p*-nitrophenol in ethylacetate at -5°. The precipitated dicyclohexylurea (DCU) is removed by filtration and then the filtrate was evaporated to obtain the desired crude ester (V) which recrystallized several times from ethanol.

Phthalylamino acid derivatives of cefaclor methyl ester (VI-VIII) were synthesized using the acid chloride method [29]. Treatment of the phthalylamino acids with PCl_5 in dry benzene converts them into the corresponding phthalylaminoacyl chlorides. Interaction of the latter intermediates with cefaclor methyl ester hydrochloride (II) proceeds with the formation of the desired products (VI-VIII). The coupling reaction was performed in the presence of two molar equivalents of a base to liberate the free cefaclor and to neutralize HCl librated during the coupling reaction. Some new derivatives of phthalyl- and tosylamino acid cefaclor (IX-XIV) were prepared through coupling reaction between cefaclor (I) and phthalyl- or tosylaminoacyl chloride respectively using the acid chloride method. The products were isolated, purified and obtained in high yield.

Using the same procedures applied in preparation of phthalylamino acid derivatives of cefaclor methyl ester (IX-XIV), we able to synthesize a new series of phthalyl- or tosyldipeptidyl cefaclor derivatives (XV-XX) via the reaction of cefaclor (I) with the requisite phthalyl- or tosyldipeptidyl chloride.

Amino acid methyl ester derivatives of *N*-Tos-cefaclor (XXI-XXV) were successfully prepared using

the phosphorus oxychloride method[29]. The desired products were obtained upon treatment a mixture of *N*-Tos-cefaclor (IV) and an amino acid ester in anhydrous THF containing excess of TEA at –15°C, with $POCl_3$. This procedures leads to high yield products with high degree of purity prior to crystallization.

Finally, two *N*-Tos-cefaclor amino acid derivatives (XXVI, XXVII) were easily synthesized using the active ester method [30] that includes the aminolysis reaction of *N*-Tos-cefaclor *p*-nitrophenyl ester (V) as mediated compound by free amino acid in aqueous sodium carbonate solution at room temperature. Formation of the desired products proceeds with rapid liberation of the *p*-nitrophenol portion of the active ester moeity. The structure of compounds (II-XXVII) was confirmed on the bases of their elemental analysis, chromatographic studies, and spectral data (Table 1).The above reactions were summarized in scheme 1.

Table 1: The spectral data of the synthesized compounds (II-XXVII).

Compd.No.	Spectral Data
II	IR (ν in cm^{-1}): 3400-3200 (broad band of amine hydrochloride interfered with OH and NH bands), 3027 (CH, aromatic), 2952, 2926 (CH, aliphatic), 1762 (C=O, β-lactam), 1686 (amide I) 1560, 1515 (C=C, aromatic), 1242, (ester, -C-O) ans 698 (C-Cl). ^{1}HNMR at δ(ppm): 3.14 (s, 2H, $CH_{2\,(2)}$), 3.72 (s, 3H, OCH_3), 4.81 (d, 1H, $CH_{(6)}$), 4.93 (s, 1H, $CH_{(11)}$), 5.68 (d, 1H, $CH_{(7)}$), 6.77-7.30 (m, 5H, Ar-H), 8.43 (br., 2H, NH), 10.51 (br., 1H, NH_3^+ canceled with D_2O).
III	IR:3350-3243 (NH, NH_2), 3036 (CH, aromatic), 2991 (CH, aliphatic), 1753 (C=O, β-Lactam), 1682 ((C=O, amide I), 1610 (C=C, ethylenic), 702(C-Cl). Ms at m\e, (% abundance): molecular ion peak at m/e = 381 is compatible with its proposed structure with a base peak at m/e = 56 (100%) in support of the proposed structure. Other significant peaks were observed in the spectrum at m/e: 281 (3.2%), 224 (32.7%), 143 (29.5%), 99 (29%), 91 (35.7%), 70 (22.1%).
IV	IR:3278 (broad bands, OH, NH), 3060 (CH, aromatic) 2973 (CH, aliphatic), 1782 (C=O, β-lactam), 1723 (C=O, acid), 1654, 1542 (amide I and II), 1348, 1162 (S=O), 814 (*p*-disubstituted benzene), 696 (C-Cl, sharp). ^{1}H-NMR: 2.32 (s, 3H, CH_3), 3.2 (s, 2H, $CH_{2(2)}$), 5.01 (s, 1H, $CH_{(6)}$), 5.17 (d, 1H, $CH_{(11)}$), 5.46 (d, 1H, $CH_{(7)}$), 7.1-7.8 (m, 9H, Ar-H), 8.50 (2H,-CONH,-SO_2NH), 9.87 (s,1H,COOH canceled with D_2O).
V	IR : 3248 (NH), 3058 (CH, aromatic), 2934 (CH, aliphatic), 1776 (C=O, β-lactam), 1678 (C=O, amide I), 1514 (C=C, aromatic), 1334, 1158 (SO_2), 698 (C-Cl). ^{1}H-NMR : 2.34 (s, 3H, CH_3), 3.11 (s, 2H, $CH_{2(2)}$), 4.98 (s, 1H, $CH_{(6)}$), 5.21 (d, 1H, $CH_{(11)}$), 5.64 (d, 1H, $CH_{(7)}$), 6.88-8.08 (m, 9H, Ar-H), 8.62 (s,1H, SO_2NH).
VI	IR: 3299 (NH), 3010, (CH, aromatic), 2952 (CH, aliphatic), 1773, 1719, 1651 (C=O of β-lactam, C=O of phthalyl, ester, and amide I respectively), 698, (C-Cl).
VII	IR: 3381, 3286 (broad bands, NH), 1776 (C=O, β-lactam) 1711 (C=O, ester), 1643 (amide I).
VIII	MS: 657 (M-1, 0.07%), 626 (0.07%), 599 (0.08%), 466 (0.17 %), 383 (0.21%), 295 (2.75%), 205 (17.61%), 148 (100%), 91 (37.92%).
IX	^{1}H-NMR: 3.16 (s, 2H, $CH_{2(2)}$), 3.89 (s, 2H, CH_2CO), 5.07 (d, 1H, $CH_{(6)}$), 5.20 (s, 1H, $CH_{(11)}$), 5.58 (d, 1H, $CH_{(7)}$), 6.94-7.83 (m, 9H, Ar-H)], 8.62 (s,1H, CONH).
X	IR: 3292 (broad bands NH, OH), 3051 (CH-aromatic), 2951 (CH-aliphatic), 1772 (C=O, β-lactam), 1716 (C=O, anhydride), 1696 (C=O, carboxylic & amide), 1636 (COO^-, carboxylate), 1540 (C=C, aromatic), 1386 (COO^-, (sym)), 720 (C-Cl).
XI	IR: 3272 (broad bands NH, OH), 3046 (CH-aromatic), 2984 (CH-aliphatic), 1772 (C=O, β-lactam), 1750, 1698 (C=O, carboxylic & amide), 1636 (COO, carboxylate), 1540 (C=C, aromatic), 1386 (COO^-, (sym)), 704 (C-Cl).
XII	IR: 3304, 3275 (broad bands of NH, OH), 3054 (CH-aromatic), 2975 (CH-aliphatic), 1784 (C=O, β-lactam), 1716 (C=O, acid), 1640, 1540(amide I and II), 1368 (COO^-, (sym)), 1328,1160 (S=O), 822 (*p*-disubstituted benzene), 672 (C-Cl sharp). ^{1}H-NMR: 2.31 (s, 3H, CH_3), 3.12 (s, 2H, $CH_{2(2)}$), 4.95 (d, 1H, $CH_{(6)}$), 5.26 (s, 1H, $CH_{(11)}$), 5.60 (d, 1H, $CH_{(7)}$), 7.04-7.93 (m, 9H, Ar-H), 8.62 (s,1H, CONH), 9.34 (s,1H, SO_2NH canceled with D_2O).
XIII	IR: 3292 (broad bands, OH, NH), 3048 (CH, aromatic), 2971 (CH, aliphatic), 1772(C=O, β-lactam), 1636, 1540 (amide I and II), 1320, 1156 (S=O), 816 (*p*-disubstituted benzene), 698 (C-Cl).
XIV	IR: 3292 (broad bands, NH, OH), 3056, 3031 (CH, aromatic), 2982 (CH, aliphatic), 1772 (C=O, β-lactam), 1716 (C=O, acid), 1636, 1540 (amide I and II), 1361 (S=O), 720 (=CH, aromatic), 693 (C-Cl).
XV	IR:3304 (broad bands, OH and NH), 3065 (CH, aromatic), 2928 (CH, aliphatic), 1776, 1728, 1648 (C=O of β-lactam, C=O of COOH and amide respectively), 698 (C-Cl sharp). ^{1}H-NMR: 3.16 (s, 2H, $CH_{2\,(2)}$), 3.86 (s, 2H, CH_2), 4.35 (s, 2H, CH_2), 5.08 (d, 1H, $CH_{(6)}$), 5.62 (s, 1H, $CH_{(11)}$), 5.68 (d, 1H, $CH_{(7)}$), 7.29-7.91 (m, 9H, Ar-H), 9.04, 9.06, 9.39, 9.42 (4H, 3NH, OH).
XVI	MS: 637 (M-2, 12.50 %), 615 (11.11 %), 591 (16.67 %), 572 (18.06 %), 451 (22.22 %), 345 (33.33 %), 288 (38.89 %), 233 (47.42 %), 163 (54.17 %), 97 (50.00 %), 51(8.11 %).
XVII	IR: 3290 (broad bands NH, OH), 3031, 1540 (CH-, and C=C aromatic), 2943(CH-aliphatic), 1792,1775 (phthalyl C=O, β-lactam C=O), 1654 (amide I), 1386 (COOH Sym), 700 (C-Cl Sharp).
XVIII	IR: 3321 (broad bands, OH, NH), 3011 (CH, aromatic), 2958 (CH, aliphatic), 1771 (C=O, β-lactam), 1716 (C=O, acid), 1683, 1558 (amide 1 and II), 1396, 1116 (S=O).
XXII	IR: 3342 (NH), 3062 (CH-aromatic), 2975, 2909 (CH-aliphatic), 1774 (C=O, β-lactam), 1728 (C=O, ester) 1663, 1542

	(amide I), 1376, 1160 (S=O), 816 (*p*-disubstituted benzene), 700 (C-Cl, sharp). ^{1}H-NMR:2.37 (s, 3H, CH_3), 3.07(d, 2H, CH_2), 3.37 (t, 2H, CH_2CO), 3.74(s,3H,OCH_3), 4.05(t,2H, CH_2NH), 5.02(d, 1H, $CH_{(6)}$), 5.26(s, 1H, $CH_{(11)}$), 5.50(d, 1H, $CH_{(7)}$), 6.88-7.72 (m, 9H, Ar-H), 8.48, 9.74 (s, NHCO, and SO_2NH canceled by D_2O).
XXIII	IR: 3284 (NH), 3060 (CH, aromatic), 2982, 2932 (CH, aliphatic) 1782 (C=O, β-lactam), 1671, 1548 (amide I and II), 1348, 1161 (S=O), 814 (*p*-disubstituted benzene).
XXIV	IR: 3254 (NH), 3066 (CH, aromatic), 2973, 2954 (CH, aliphatic) 1758 (C=O, β-lactam), 1740 (C=O, ester) 1656, 1543 (amide I and II), 1618, 1508 (C=C, aromatic), 1350, 1164 (S=O), 814 (*p*-disubstituted benzene), 700 (C-Cl).
XXV	IR: 3282 (NH), 3061 (CH, aromatic), 2972 (CH, aliphatic), 1782 (C=O, β-lactam), 1723 (C=O, ester) 1654, 1542 (amide I and II), 1602 (C=C, aromatic), 1312, 1160 (S=O), 831 (*p*-disubstituted benzene), 696 (C-Cl sharp). ^{1}H-NMR: 1.92 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 5.18(s, 1H, $CH_{(11)}$), 5.57 (d, 1H, $CH_{(7)}$), 6.81-7.89 (m, 13H, Ar-H), 8.28 (s,1H,NHCO-), 9.59 (s,1H,-SO_2NH canceled by D_2O).
XXVI	IR: 3282 (broad bands, OH, NH), 3062 (CH, aromatic), 2932 (CH, aliphatic), 1768-1654 (C=O), 1514 (C=C, aromatic), 1334 (SO_2), 698 (C-Cl, sharp). ^{1}H-NMR : 2.2 (s, 3H, CH_3), 2.95 (d, 2H, CH_2), 3.3 (s, 2H, $CH_{2(2)}$), 4.1 (t, 1H, CH), 4.9 (d, 1H, $CH_{(6)}$), 5.5 (d, 1H, $CH_{(11)}$), 5.9 (d, 1H, $CH_{(7)}$), 6.72-7.92 (m, 13H, Ar-H), 11.43 (s, 1H, COOH), canceled by D_2O].
XXVII	IR: 3297 (broad bands, OH, NH), 3061 (CH, aromatic), 2957, 2926 (CH, aliphatic), 1776 (C=O of β-lactam), 1736 (C=O, acid), 1681 (amide I), 1525 (C=C, aromatic), 1329, 1159 (SO_2), 699 (C-Cl, ,sharp).

Antimicrobial screening results

Antibiogram susceptibility and resistance of twenty seven semi-synthetic antibiotics from cefaclor:

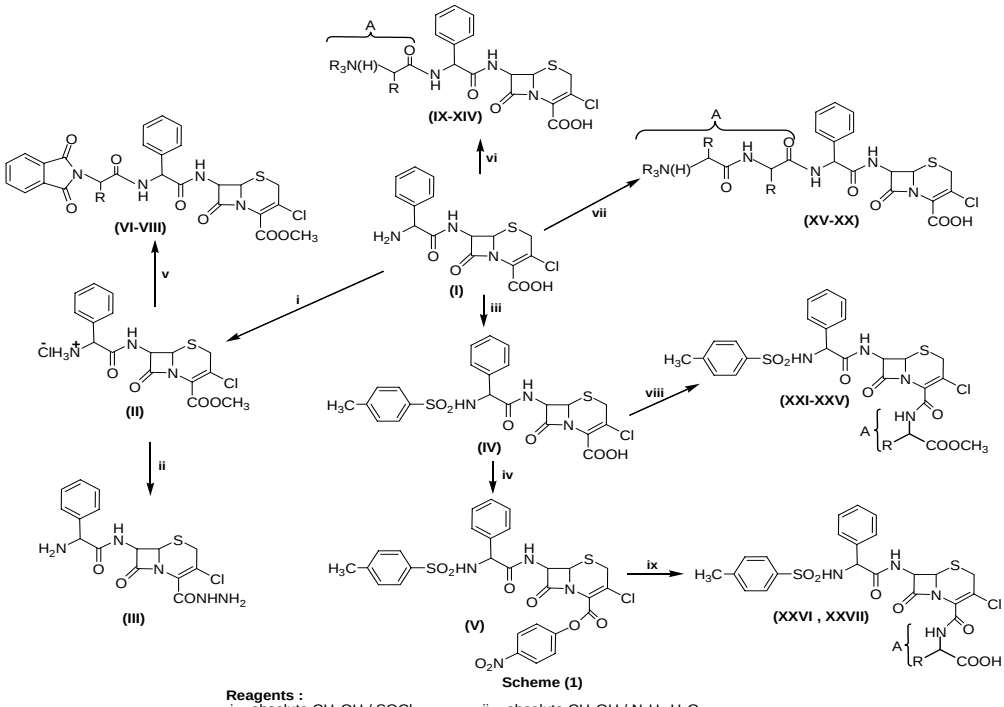
Twenty seven semi-synthetic antibiotics or derivatives synthesized from cefaclor were applied during this study against the growth of the six bacterial and three unicellular and multicellular fungal strains by paper disc diffusion method [31,33]. All antimicrobial activities of the new synthesized derivatives or semi-synthetic antibiotics were compared with the activities of the starting cefaclor antibiotic. It was clear from the results recorded in tables (2) and (3) that cefaclor (I) exhibited susceptibility or activity against only four tested microorganisms only viz. Bacillus subtilis NCTC 10400 (32mm), Staphylococcus aureus ATCC 25923 (36mm), Escherichia coli ATCC 25922 (23mm) and Candida albicans (35mm) represented Gram-positive bacilli, Gram-positive staphylococci, Gram-negative short rods and unicellular fungi respectively.

Cefaclor derivatives or semi-synthetic antibiotics IV, XII, XIII, XIV, and XVI were highly active against all tested Gram-positive and Gram-negative bacterial growth respectively. The previously mentioned five cefaclor derivatives exhibited broad spectrum antibacterial activity against the tested bacterial strains. Cefaclor derivatives or semi-synthetic antibiotics XII, XIII and XIV were highly active against all tested bacterial strains i.e. Bacillus subtilis NCTC 10400 (35, 30 and 34 mm), Staphylococcus aureus ATCC 25923 (40, 32 and 40 mm), Enterococcus faecalis NCTC 821(33, 29 and 33 mm), Escherichia coli ATCC 25922 (34, 30 and 35 mm), Proteus vulgaris NCTC 4175 (30, 27 and 32 mm), Pseudomonas aeruginosa ATCC 10415 (29, 25 and 30 mm) respectively. Cefaclor derivatives or semi-synthetic antibiotics IV and XVI were moderately active against all tested bacterial strains i.e. Bacillus subtilis NCTC 10400 (26 and 27 mm), Staphylococcus aureus ATCC 25923 (28 and 30 mm), Enterococcus faecalis NCTC 821(23 and 26 mm), Escherichia coli ATCC 25922 (25 and 28 mm), Proteus vulgaris NCTC 4175 (20 and 28 mm), Pseudomonas aeruginosa ATCC 10415 (20 for IV) respectively. Five semi-synthetic antibiotics synthesized from cefaclor IX, XI, XV XVII and XXI exhibited weak to moderately activity against some tested bacterial strains i.e., Bacillus subtilis NCTC 10400 (20,15,21,29 and 16 mm), Staphylococcus aureus ATCC 25923 (26 ,20, 25, 21 and 19 mm), Enterococcus faecalis NCTC 821(15 and 15 mm for IX and XVII respectively) and Escherichia coli ATCC 25922 (19, 14, 17, 20 and 13 mm) , Proteus vulgaris NCTC 4175 and Pseudomonas aeruginosa ATCC 10415 were resistant of previously mentioned five derivatives. Unfortunately the remaining semi- synthetic antibiotics were found to be antibacterial inactive. Cefaclor (I) , (XII) and XIII were highly active against unicellular fungi i.e. Candida albicans (36 35 and 33 mm respectively), while other five semi-synthetic antibiotics IX, X, XIV, XVII and XXIII were exhibited low activity (25, 27 , 25, 24 and 22 mm) respectively in comparable with standard cefaclor (Table 3). All semi-synthetic cefaclor antibiotics were found to be completely inactive with all tested filamentous fungi viz. Aspergillus niger and Aspergillus flavus. Results recorded in table (4) showed concentration (*MIC*, mg/ml) of the most active compounds. This study showed that :

1) Esterification and hydrazinolysis, reactions of cefaclor completely destroyed the antimicrobial activity of cefaclor.

2) Introduction of tosylaminoacyl moieties in combination with cefaclor (XII, XIII and XIV) and *N*-Tos-cefaclor (IV) improved and verified the antibacterial activity of cefaclor especially against the strains of gram-negative bacteria.

3) The remaining derivatives of cefaclor resulting from its reactions with phthalylaminoacyl- , dipeptidyl- , amino acid methyl esters or free amino acids decreased or canceled, in most cases the biological activity of cefaclor.



Scheme (1)

Reagents :
i = absolute CH_3OH / $SOCl_2$, ii = absolute CH_3OH / $N_2H_4{\cdot}H_2O$
iii = *p*-tosyl chloride / mixture of H_2O, THF - TEA , iv = *p*-nitrophenol / DCC / CH_3CO_2Et
v = phthalylaminoacyl chloride / mixture of THF and DMF - TEA
vi = phthalyl or tosylaminoacyl chloride / mixture of THF and DMF - TEA
vii = phthalyl or tosyldipeptidyl chloride / THF - TEA
viii = amino acid methyl ester hydrochloride / THF-TEA / $POCl_3$
ix = amino acid / Na_2CO_3 soln.

Table (1): The physical data of the synthesized cefaclor derivatives (II-XXVII).

Compd No.	A	M.P. [°C]	Yield %	Cryst. solv*	R_f	Mol. formula	Elemental analysis % calc./found		
							C	H	N
II	---	64	97	A	0.93	$C_{16}H_{17}Cl_2N_3O_4S$ 418	45.93 45.74	4.06 3.85	10.04 10.28
III	---	227	84	B	0.63	$C_{15}H_{16}ClN_5O_3S$ 381.5	47.18 47.41	4.19 4.26	18.35 18.09
IV	---	183	86	C	0.73	$C_{22}H_{20}ClN_3O_6S_2$ 521.5	50.62 50.38	3.83 3.86	8.05 8.28
V	---	80	71	B	0.77	$C_{28}H_{23}ClN_4O_8S_2$ 642.5	52.29 52.41	3.57 3.39	8.71 8.62
VI	Pht-Gly.	135-37	81	B	0.65	$C_{26}H_{21}ClN_4O_7S$ 568.5	54.88 54.90	3.69 3.70	9.85 9.87
VII	Pht-β-Ala.	161-162	79	B	0.71	$C_{27}H_{23}ClN_4O_7S$ 582.5	55.62 55.60	3.94 3.91	9.61 9.62
VIII	Pht-L-Phe.	170	68	B	0.69	$C_{33}H_{27}ClN_4O_7S$ 658.5	60.13 60.11	4.10 4.00	8.50 8.48
IX	Pht-Gly.	185-87	69	B	0.67	$C_{25}H_{19}ClN_4O_7S$ 554.5	54.10 54.19	3.42 3.23	10.09 9.89
X	Pht-β-Ala.	178-79	82	D	0.71	$C_{26}H_{21}ClN_4O_7S$ 568.5	54.88 54.79	3.69 3.71	9.85 9.71
XI	Pht-L-Phe.	170-72	79	B	0.69	$C_{32}H_{25}ClN_4O_7S$ 644.5	59.58 59.44	3.87 4.08	8.68 8.60
XII	Tos-Gly.	173-76	84	C	0.68	$C_{24}H_{23}ClN_4O_7S_2$ 578.5	49.78 49.93	3.97 4.13	9.68 9.78
XIII	Tos-β-Ala.	143-44	76	C	0.63	$C_{25}H_{25}ClN_4O_7S_2$ 592.5	50.63 50.82	4.21 4.29	9.45 9.65
XIV	Tos-L-Phe.	215-16	71	D	0.77	$C_{31}H_{29}ClN_4O_7S_2$ 668.5	55.64 55.76	4.33 4.12	8.37 8.53
XV	Pht-Gly. Gly.	260	79	D	0.83	$C_{27}H_{22}ClN_5O_8S$ 611.5	52.98 52.99	3.59 3.45	11.44 11.31
XVI	Pht-β-Ala. β-Ala.	98	74	D	0.81	$C_{29}H_{26}ClN_5O_8S$ 639.5	54.41 54.34	4.06 4.27	10.94 10.88
XVII	Pht-L-Phe. L-Phe.	173	78	D	0.87	$C_{41}H_{34}ClN_5O_8S$	62.16	4.29	8.84

						791.5	62.11	4.47	8.71
XVIII	Tos-Gly. Gly.	176	74	E	0.65	$C_{26}H_{26}ClN_5O_8S_2$ 635.5	49.09 49.24	4.09 3.85	11.01 10.91
XIX	Tos-β-Ala.β-Ala.	182	72	E	0.62	$C_{28}H_{30}ClN_5O_8S_2$ 663.5	50.64 50.61	4.52 4.35	10.55 10.69
XX	Tos-L-Phe.L-Phe.	185	73	E	0.70	$C_{40}H_{38}ClN_5O_8S_2$ 815.5	58.86 58.66	4.65 4.74	8.58 8.43
XXI	Gly-OMe	145-148	82	D	0.71	$C_{25}H_{25}ClN_4O_7S_2$ 592.5	50.63 50.50	4.21 3.96	9.45 9.72
XXII	β-Ala-OMe	149-151	79	D	0.69	$C_{26}H_{27}ClN_4O_7S_2$ 606.5	51.44 51.31	4.45 4.67	9.23 9.14
XXIII	L-Phe-OMe	211-213	84	C	0.68	$C_{32}H_{31}ClN_4O_7S_2$ 682.5	56.26 56.47	4.54 4.22	8.20 8.13
XXIV	L-Pro-OMe	159-160	76	C	0.63	$C_{28}H_{29}ClN_4O_7S_2$ 632.5	53.12 53.34	4.58 4.71	8.85 9.02
XXV	p-ABA-OMe	220-222	71	D	0.77	$C_{30}H_{27}ClN_4O_7S_2$ 654.5	55.00 55.27	4.12 4.39	8.55 8.34
XXVI	L-phe	129-131	63	D	0.70	$C_{31}H_{29}ClN_4O_7S_2$ 668.5	55.64 55.86	4.33 4.20	8.37 8.25
XXVII	L-Tyr	180-183	68	E	0.86	$C_{31}H_{29}ClN_4O_8S_2$ 684.5	54.34 54.53	4.23 4.45	8.18 8.02

*Crystallization solvent: A= Methanol-diethyl ether; B= Ethanol C= Ethanol-water; D= dioxane; E = DMF-water.

Table 3. In-vitro antimicrobial activities of some synthestic cefaclor derivatives (I-XXVII).

Compd No.	Mean diameter of inhibition zone(mm)					
	B.subtilis NCTC10400	S.aureus TCC25923	E.faecalis NCTC 821	E.coli ATCC25922	P.vulgaris NCTC4175	P.aeruginosa ATCC10425
I	32	36	0	23	0	0
II	0	0	0	0	0	0
III	0	0	0	0	0	0
IV	26	28	23	25	20	20
V	0	0	0	0	0	0
VI	0	0	0	0	0	0
VII	0	0	0	0	0	0
IX	20	26	15	19	0	0
X	0	0	0	0	0	0
XI	15	20	0	14	0	0
XII	35	40	33	34	30	29
XIII	30	32	29	30	27	25
XIV	34	40	33	35	32	30
XV	21	25	0	17	15	0
XVI	27	30	26	28	28	0
XVII	29	21	15	20	0	0
XXI	16	19	0	13	0	0
XXII	0	0	0	0	0	0
XXIII	0	0	0	0	0	0
XXIV	0	0	0	0	0	0
XXV	0	0	0	0	0	0
XXVI	0	0	0	0	0	0
XXVII	0	0	0	0	0	0

Table 3. In-vitro antifungal activities of some synthetic cefaclor derivatives (I-XXVII).

Compd. No.	Mean diameter of inhibition zone(mm)		
	Candida albicans	Aspergillus niger	Aspergillus flavus
I	35	0	0
II	0	0	0
IX	25	0	0
X	27	0	0
XI	0	0	0
XII	35	0	0
XIII	33	0	0
XIV	25	0	0
XVI	0	0	0
XVII	24	0	0
XXIII	22	0	0

Table 4. Minimum inhibitory concentration (*MIC*) of most active cefaclor derivatives.

Comp.No.	Minimum inhibitory concentration (*MIC*) (mg/ml)					
	B.subtilis NCTC10400	S.aureus TCC25923	E.faecalis NCTC 821	E.coli ATCC25922	P.vulgaris NCTC4175	P.aeruginosa ATCC10425
XII	0.005	0.003	0.01	0.015	0.02	0.02
XIII	0.007	0.005	0.019	0.022	0.027	0.03
XIV	0.006	0.004	0.017	0.02	0.025	0.024
XVI	0.01	0.007	0.017	0.021	0.027	0.027

Experimental

Melting points were uncorrected and measured on electric melting point apparatus SMPI. Purity of compounds was checked by thin layer chromatography (TLC) on plastic sheets coated with silica gel 60 (Merck) and developed with n-butanol: acetic acid: water (4: 1: 1) using Iodine-potassium Iodide (20%) and benzidine solutions as spraying agents, and also detected under UV lamp. The infrared, IR, spectra (ν max, $cm^{-1)}$ were taken in KBr discs using FTIR-2000 instrument. The Nuclear Magnetic Resonance, ^{1}HNMR spectra were measured in DMSO-d_6 or $CDCl_3$ using FX90Q Fourier Transform NMR spectrometer. The mass spectra were performed using Shimad Zu-GC-MS-QP 1000 EX using the direct inlet system. Elemental analyses were carried out at Microanalytical Unit, Faculty of Science, and Cairo University. The biological activities were measured in Department of Potany, Faculty of Science, Al-Azhar University, and Cairo, Egypt.

1. Cefaclor (I) was supplied from Cairo Pharmaceutical and Chemical Industrial Company, Cairo, Egypt.

2. Synthesis of cefaclor methyl ester hydrochloride (II).

Cefaclor (I, 0.01 mol) was added to 100 ml of abs. methanol and the mixture was cooled in an ice bath at (0-5°C) then (0.011 mol.) of pure thionyl chloride was added dropwise. The temperature of the reaction was kept below (5°C) during the addition of thionyl chloride. The reaction was stirred for additional 3 hrs at 5-10°C, kept overnight at room temperature and then the solvent was removed in vacuo. Methanol was added and re-evaporated several times.

3. Synthesis of cefaclor hydrazide (III).

To a solution of cefaclor methyl ester hydrochloride (II**,** 0.01 mole) in 100 ml abs. methanol was added (0.015 mol) of hydrazine hydrate and the solution was left for 24 hrs at room temperature. The hydrazide compound was filtered and recrystallized from ethanol.

4. Synthesis of *N*-Tos-cefaclor (IV).

A solution of *p*-tosylchloride (0.01 mol) in 10 ml of THF was added over a period of 30 min. to a stirred, cooled suspension of cefaclor (I, 0.01 mol) in a mixture of 8 ml. of water, 4 ml. of THF, and triethylamine (0.011 mol). The mixture was stirred for an additional 45 min. at room temperature, concen-trated under reduced pressure and 10 ml of water was then added. The solution obtained was washed with ether and the product precipitates on acidification of the aqueous layer with dil. HCl. The crude product was recrystallized from aqueous ethanol.

5. Synthesis of *N*- Tos-cefaclor *p*-nitophenyl ester (V).

A solution of *N*-cefaclor **(**IV, 0.01mol) and *p*-nitro-phenol (0.01 mol) in ethyl acetate (25 ml) was stirred and cooled in an ice-bath to -5°C and dicyclohexyl-carbodiimide (DCC; 0.01mol) was then added, in a few portions. The reaction mixture was stirred at 0°C for 3 hrs and the left to stand at room temperature for overnight. The precipitated dicyclohexylurea (DCU) was removed by filtration and the filtrate was evaporated under reduced pressure. The residual material was dissolved in ethylacetate and few drops of gl. AcOH was added and the solution left for 24 hrs at room temperature. The solution was filtered again to remove the precipitated DCU crystals. The filtrate was re-evaporated under vacuum and the residual material was purified by recrystallization many times from 95% ethanol.

6. General procedure for Synthesis of Phthalyl amino acid derivatives of cefaclor methyl ester (VI-VIII).

A solution of phthalylaminoacyl chloride of (0.001 mol) in 10 ml of THF was added over a period for 30 min. to a stirred, cooled suspension of cefaclor methyl ester hydrochloride (II, 0.0011 mol), previously stirred and treated with triethylamine (0.0022 mol) in a mixture of 20 ml THF and 5 ml DMF. The mixture was stirred for an additional 3 hrs at room temperature and then poured into crushed iced-water to extract triethylamine HCl. The crude product that precipitated, removed by filtration, washed with cold water and then recrystallized from ethanol.

7. General procedure for Synthesis of Phthalyl- or tosylamino acid derivatives of cafaclor (IX-XIV).

To a cold solution of cefaclor (I, 0.001 mol) in 20 ml of THF and 5 ml of DMF containing triethylamine (0.001 mol) was slowly added a solution of phthalyl- or tosylaminoacyl chloride (0.001 mol) in 10 ml of THF. The reaction mixture was stirred for 1 hr at 5°C and 3 hrs at room temperature, and then poured into crushed ice water. The precipitated crude products were isolated, dried and purified by recrystallization from the proper solvent.

8. General procedure for Synthesis of Phthalyl- or tosyldipeptide cefaclor derivatives (XV-XX).

A solution of phthalyl- or tosyldipeptidyl chloride (0.001 mol) in 20 ml of THF was added dropwisely over a period of 30 min. to a stirred, cooled suspen-sion of cefaclor (I, 0.001) in 20 ml of THF containing triethylamine (0.0011 mol). The remaining procedure was the same for that used for preparation of (IX-XIV). The isolated dipeptide derivatives were recrystallized from the proper solvent.

9. General procedure for Synthesis of *N*-Tos-cefaclor amino acid methyl ester derivatives (XXI-XXV).

A mixture of *N*-Tos-cefaclor (IV, 0.002 mol), an amino acid methyl ester hydrochloride (0.0022 mol) and triethylamine (0.0022 mol) suspended in 20 ml. of anhydrous THF was cooled to -15^{0}C with shaking for 15 minutes. The mixture was then treated with purified phosphorus oxychloride (0.003 mol) and directly thereafter (0.004 mol) of triethylamine was added. After the reaction mixture has stood for 1 hr. at -15°, 20 ml. of water was added and the mixture evaporated in vacuo in order to the remove tetrahydrofuran. The residual material was treated with 20 ml. of water, extracted two times with 20 ml. portion of ethyl acetate, and the combined ethyl acetate extracts were washed three times each with 5 ml. portions of water, with several portions of 5% sodium bicarbonate solution, and finally with water. After being dried with anhydrous sodium sulfate, the ethyl acetate fraction was concentrated to dryness at room temperature. The residual compounds were recrystallized from the proper solvent.

10. General procedure for Synthesis of *N*-Tos-cefaclor amino acids (XXVI and XXVII).

A solution of an amino acid (0.001 mol) in Na_2CO_3 solution (0.002 mol) was treated portionwisely with a solution of *N*-Tos-cefaclor *p*-nitrophenyl ester (V, 0.001 mol) in THF. The mixture was stirred at room temperature for 3 hrs, and then the mixture was poured into crushed ice water, and acidified with dil. hydrochloric acid. The resulting precipitate was filtered, washed many times with 10 ml portions of water, dried and then purified by recrystallization from the proper solvent.

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Interactive Compromise Stability of Multi-objective Nonlinear Programming problems

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Abstract: This paper presents a solution method for multi-objective nonlinear programming (MONLP) problems and stability of this solution. The method, offers a practical solution to MONLP problems by deriving the compromise weights and combining judgment with an automatic optimization technique in fuzzy decision making. This is achieved by using the method and algorithm of compromise programming and the method of compromise weights and we obtain the stability for the solution in each step of the algorithm. A numerical example illustrates various aspects of the results developed in this paper. A maple procedure for this algorithm is introduced.

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Keywords: MONLP; Stability; Interactive decision making; Compromise weights; Membership functions**.**

1. Introduction

Most decision problems have multiple objectives conflicting among themselves. The solution for such problems can only be obtained by trying to get compromises based on information provided by the decision maker (**DM**). Several methods have been developed to solve multiobjective decision making (**MODM**) problems, see [10]. In [5,8] some of these methods are based on prior information required from the **DM**. This information may be in the from the desired achievement levels of the objective functions and the ranking of the levels indicating their importance, such as in goal programming . It may also be in the form of weights showing the importance of the objectives. The disadvantages with these method is that the **DM** cannot easily provided this prior information since he has no idea about the solution process of the problem. Other methods, called interactive methods, have been developed in order to overcome this disadvantage. There are two categories of interactive methods. Interactive methods of the first type require the **DM** to provide some trade-offs among the attained values of the objective functions in order to determine the new solution [4]. The interactive methods of the second type require the **DM** to provide some preference information by comparing the various efficient solutions in the space of the objective functions or the decision variables . The quantity and complexity of the information required from the **DM** in such methods are important factors affecting the chances of reaching the best compromise solution. In [3, 7] an interactive linear multiple objective method, called interactive compromise programming (**ICP**) were introduced. The notions of the solvability set, stability set of the first kind and stability set of the second kind, and analysed these concepts for parametric convex nonlinear programming problems were introduced in [6, 9].

This paper presents an interactive stability compromise programming method for solving **MONLP** problems by using the compromise weights from the pay-off table and fuzzy membership function for each objective function. An illustrative example is given to clarify the obtained results.

2. Problem Formulation

Let us consider the **MONLP** problem:

$$(\text{MONLP}): \quad \max\left(f_1(x), f_2(x), ..., f_m(x)\right)$$

subject to

$$x \in X = \left\{x \in R^n \mid g_j(x) \le 0, j = 1,2,...,k\right\}$$

where $f_i(x)$, $i = 1,...,m$, and $g_j(x)$, $j = 1,...,k$, are convex real valued functions which belong to class $C^{(1)}$.

The corresponding scalarization problem is

$$(\mathbf{MONLP})_\lambda \qquad \max \sum_{i=1}^{m} \lambda_i f_i(x)$$

$$\text{subject to } x \in X,$$

where $\lambda = (\lambda_1,...,\lambda_m) \neq 0, \lambda_i \ge 0, i = 1,2,...,m$, and $\sum_{i=1}^{m} \lambda_i = 1.$

Let $f_i(x)$ be the *i*th objective function and $f_i^U(x)$ be the maximum possible values of $f_i(x)$ and $f_i^L(x)$ are the minimum possible values of $f_i(x)$ found under the constraints, respectively . To obtain the compromise solution of the MONLP problem , find the solution which has a minimum distance with respect to the ideal solution $f_i^U(x)$. This requires normalization of the objective functions and appropriate choice for the distance measure. The solution found in this way is a reduced set of all efficient solution . The set of compromise solution may be large, and also the choice of weights by the DM may be difficult.

these difficulties could be reduced by combining the basic ideas for the methods of compromise programming and compromise weights .

3. Compromise Weights

Here, we introduce a method based on the following two main ideas:

First, the DM could state his preference among some alternative solutions more easily if the values of objective functions were measured on the some scale varying between zero and one . This could be done by employing "the membership function for the objective functions" concept in the compromise programming. In order to elicit a membership function $\mu_{f_i}(x)$ from the DM for each of the objective functions $f_i(x)$ in MONLP problems, we first calculate the individual minimum f_i^L and maximum f_i^U of each objective function $f_i(x)$ under the given constraints. By taking account of the calculated individual minimum and maximum of each objective function together with the rate of increase of membership of satisfaction**,** **DM** must determine his subjective membership function $\mu_{f_i}(x)$ which is a strictly monotone increasing function with respect to $f_i(x)$.

Here, it is assumed that

$$\mu_{f_i}(x)=0 \text{ or } \to 0 \text{ if } f_i(x)\le f_i^L \text{ and}$$

$$\mu_{f_i}(x)=1 \text{ or } \to 1 \text{ if } f_i(x)\ge f_i^U,$$

where f_i^a represents the value of $f_i(x)$ such that the value of membership function $\mu_{f_i}(x)$ is a.

In this method , the following definition of the membership functions is used for scaling:

$$\mu_{f_i}(x)=\frac{f_i(x)-f_i^L}{f_i^U-f_i^L}, \qquad (1)$$

where $f_i(x)$ are the objective functions, f_i^U are the maximum possible values of $f_i(x), i=1,2,...,m$ and f_i^L are the minimum possible values of $f_i(x)$ satisfying the constraints $x\in X$ .The $\mu_{f_i}(x)$ are defined as the membership functions of $f_i(x)$ to the possible value $f_i(x)$.

The corresponding scalarization problem is:

$$\max \mu_{f^{m+1}}(x)=\sum_{i=1}^{m}\lambda_i\mu_{f_i}(x) \qquad (2)$$

subject to $x\in X$.

The second main idea , one of the main drawbacks of the interactive methods is the difficulty of getting the weights of the objective functions from the **DM** even if values of objective functions are presented to him on the same scale.

In this method , the compromise weights of objective functions can be obtained by constructing the pay-off table displaying values of objective functions at $x^1,...,x^m$, where x^i solves $\max f_i(x)$, $i=1,...,m$, subject to $x\in X$. A pay-off table is

	f_1	f_2	$\cdots$	f_i	$\cdots$	f_m
f_1	f_1^*	f_1^2	$\cdots$	f_1^j	$\cdots$	f_1^m
f_2	f_2^1	f_2^*	$\cdots$	f_2^j	$\cdots$	f_2^m
$\vdots$	$\vdots$	$\vdots$		$\vdots$		$\vdots$
f_i	f_i^1	f_i^2	$\cdots$	f_i^*	$\cdots$	f_i^m
$\vdots$	$\vdots$	$\vdots$		$\vdots$		$\vdots$
f_m	f_m^1	f_m^2	$\cdots$	f_m^j	$\cdots$	f_m^*

where $f_i^*=f_i(x^i)$ and $f_i^j=f_i(x^j)$ for each $i=1,...,m$, $j=1,...,k$ and $i\neq j$ the compromise weights λ_i, $i=1,2,...,m$ can be obtained from the pay-off matrix by the formula,

$$\lambda_i=\frac{e^{\alpha a_i}}{\sum_{i=1}^{m}e^{\alpha a_i}},\ i=1,2,...,m,$$

$$\alpha=\frac{1}{a_m-a_{m-1}}\ln\left|\sum_{i=1}^{m}\frac{a_i}{a_m}\right|, a_i=\hat{f_i}-f_i^*, i=1,2,...,m. \qquad (3)$$

where $\hat{f_i}=\max f_i(x^i)$ is the maximum entry in row i.

4. Stability set of the first kind [7]

Definition 1. The solvability set of problem $(\mathbf{MONLP})_\lambda$ is defined by

$$B=\left\{\lambda\in R_+^m \mid \max_{x\in X}\sum_{i=1}^{m}\lambda_i f_i(x) \text{exists}\right\},$$

where R_+^m is the nonnegative orthant of the vector parameter λ.

Definition 2. Suppose that $B\neq\varnothing$ with a corresponding optimal point $\bar{x}$, then the stability set of the first kind of problem $(\text{MONLP})_\lambda$ corresponding to $\bar{x}$ is defined by

$$S(\bar{x})=\left\{\lambda\in B \mid \sum_{i=1}^{m}\lambda_i f_i(\bar{x})=\max_{x\in X}\sum_{i=1}^{m}\lambda_i f_i(x)\right\}.$$

It is clear that the stability set of the first kind is the set of all parameters corresponding to an optimal solution of the scalarizing problem.

Let $\bar{\lambda}\in S(\bar{x})$ then there exist $\bar{u}\in R^k$ such that $(\bar{x},\bar{u})$ solves the following Kuhn-Tucker problem:

$$\sum_{i=1}^{m}\bar{\lambda}_i\frac{\partial f_i(\bar{x})}{\partial x_\alpha}+\sum_{j\notin J}\bar{u}_j\frac{\partial g_j(\bar{x})}{\partial x_\alpha}=0,\ \alpha=1,2,...,n,$$

$g_j(\bar{x}) \le 0, \bar{u}_j g_j(\bar{x}) = 0,\ j = 1,2,...,k,$

$\bar{u}_j = 0,\ j \in J \subset \{1,2,...,k\}, \bar{u}_j \ge 0,$

$j \in \{1,2,...,k\} - J,$

that means, we order the function $g_j(x)$, j=1,2,…,k, in such a way that

$j \in \{1,2,...,s\}$ if $g_j(\bar{x}) = 0,$

$j \in \{s+1,...,k\}$ if $g_j(\bar{x}) < 0.$

Consider the system of equations

$$\sum_{i=1}^{m} \lambda_i \frac{\partial f_i(\bar{x})}{\partial x_\alpha} + \sum_{j=1}^{s} u_j \frac{\partial g_j(\bar{x})}{\partial x_\alpha} = 0, \quad \text{(I)}$$

$\alpha = 1,2,...,n.$

It represent n linear homogenous equations in $m+s$ unknowns λ_i, $i = 1,2,...,m$, and u_j, $j = 1,2,...,s$, which can be solved explicitly.

Suppose that $\lambda_i^* \ge 0,\ i = 1,2,...,m,$ and $u_j^* \ge 0,$ j=1,2,…,s, solve the above system of equations, then it is clear that $(\bar{x}, \bar{u})$ solves the Kuhn-Tucker problem , where $\bar{u}_j = u_j^*,\ j = 1,2,...,s,\ \bar{u}_j = 0,$ j=s+1,…,k, and hence $\lambda^* \in S(\bar{x})$.

Let us define the set

$$P(\lambda,u) = \left\{(\lambda,u) \in R_+^{m+s} \,\middle|\, (\lambda,u) \text{solves the system (I)}\right\},$$

where R_+^m and R_+^s are the nonnegative orthants of the R^m vector $\lambda-$space, and R^s vector $u-$space, respectively . Then

$$S(\bar{x}) = \left\{\lambda \in R_+^m \,\middle|\, (\lambda,u) \in P(\lambda,u)\right\}. \quad \text{(II)}$$

If $g_j(\bar{x}) < 0, j = 1,2,...,k$, then it is easy to see that $S(\bar{x})$ can be written in the following form:

$$S(\bar{x}) = \left\{\lambda \in R_+^m \mid \sum_{i=1}^{m} \lambda_i \frac{\partial f_i(\bar{x})}{\partial x_\alpha} = 0, \alpha = 1,2,...,n\right\}.$$

5. Interactive compromise algorithm

In this method, the solution process by solving 2m simple nonlinear programming problems to find the maximum and minimum possible values of m objective under the given constraints.

The compromise weights of the objective functions are determine from the Eq.(3) and employed in the problem (2) we have

$$\max \mu_{f^{m+1}}(x) = \sum_{i=1}^{m} \lambda_i \mu_{f_i}(x)$$

subject to $x \in X$

where $\mu_{f^{m+1}}(x)$ is the composite function of $\mu_{f_i}(x)$ and it determines the $(m+1)th$ solution .

The steps of the algorithm can be summarized as follows:

Step 1. Determine f_i^U, f_i^L for all i=1,…,m, as follows:

(i) $\max f_i(x)$

subject to $x \in X$,

The solutions of this problem are x^{iU} and f_i^U which are known as the "ideal solution".

(ii) $\min f_i(x)$

subject to $x \in X$,

The solution are x^{iL} and f_i^L which are known as the "anti -ideal solution".

Step 2. Determine the membership functions corresponding the solution x^{iU}, $i = 1,2,...,m$ as in the relation (1).

Construct the pay-off table

	f_1	f_2	⋯	f_i	⋯	f_m
f_1	f_1^*	f_1^2	⋯	f_1^j	⋯	f_1^m
f_2	f_2^1	f_2^*	⋯	f_2^j	⋯	f_2^m
⋮	⋮	⋮		⋮		⋮
f_i	f_i^1	f_i^2	⋯	f_i^*	⋯	f_i^m
⋮	⋮	⋮		⋮		⋮
f_m	f_m^1	f_m^2	⋯	f_m^j	⋯	f_m^*

where x^i solves

$\min f_i(x), i = 1,2,...,m$, $f_i^* = f_i(x^i)$

subject to $x \in X$,

$f_i^j = f_i(x^j)$ for each i=1,…,m, j=1,2,…,k, $i \ne j$ and construct fuzzy matrix.

$\mu_{f_i^j}$	x^1	x^2	...	x^m	f^u
f_1	$\mu_{f_1^1}$	$\mu_{f_1^2}$	…	$\mu_{f_1^m}$	f_1^U
f_2	$\mu_{f_2^1}$	$\mu_{f_2^2}$	…	$\mu_{f_2^m}$	f_2^U
⋮	⋮	⋮		⋮	⋮
f_m	$\mu_{f_m^1}$	$\mu_{f_m^2}$	…	$\mu_{f_m^m}$	f_m^U

Step 3: The compromise weights $\lambda_i, i = 1,2,...,m$ can be found from

$$\lambda_i = \frac{e^{\alpha a_i}}{\sum_{i=1}^{m} e^{\alpha a_i}}, i = 1,2,...,m ,$$

$$\alpha = \frac{1}{a_m - a_{m-1}} \ln \left| \sum_{i=1}^{m} \frac{a_i}{a_m} \right|, \ a_i = \hat{f}_{ii} - f_i^*, \ i = 1,2,...,m.$$

$\hat{f}_i = \max f_i(x^i)$ is the maximum entry in row i .

Step 4: By using this weights , we establish the new compromise solution x^{m+1}, from the problem (2).

Step 5. Determine the stability set of the first kind corresponding to this solution as in relations (I) and (II).

Step 6: Determine the membership objective

functions of the new solution of the problem in step 4, $\mu_{f^{m+1}}$ .Add this column to table of fuzzy in step 2.

Step 7: Ask the DM whether he prefers one solution strictly over all the other *m*-solutions if he does go to step 8 , otherwise ask him his least preferred solution among all the others. Then replace this preferred solution by the new found in step 6 and go to step 3.

Step 8: Stop.

6. Numerical example

Let us consider the following problem

$$\min f_1(x) = x_1 + x_2^2,$$

$$\min f_2(x) = (x_1 - 5)^2 + x_2,$$

$$\text{subject to } x_1^2 + x_2^2 \le 25,$$

$$x_1 \ge 0, \quad x_2 \ge 0.$$

The solution of this example will be obtained using a Maple program:

Step1.

(I) $\max f_1(x) = x_1 + x_2^2$,

subject to $x_1^2 + x_2^2 \le 25,$

$x_1 \ge 0, \quad x_2 \ge 0.$

solution $x^{1U} = (0.5, 4.97), \; f_1^U = 25.25$.

(II) $\max f_2(x) = (x_1 - 5)^2 + x_2^2,$

subject to $x_1^2 + x_2^2 \le 25,$

$x_1 \ge 0, \quad x_2 \ge 0.$

solution $x^{2U} = (0,5), \quad f_2^U = 30$.

(III) $\min f_1(x) = x_1 + x_2^2$

subject to $x_1^2 + x_2^2 \le 25,$

$x_1 \ge 0, \quad x_2 \ge 0.$

solution $x^{1L} = (0,0), \quad f_1^L = 0$.

(IV) $\min f_2(x) = (x_1 - 5)^2 + x_2^2,$

subject to $x_1^2 + x_2^2 \le 25,$

$x_1 \ge 0, \quad x_2 \ge 0.$

solution $x^{2L} = (5,0), \quad f_2^L = 0$.

Step 2.the corresponding pay- off table is

	f_1	f_2
f_1	0	5
f_2	25	0

where $f_1(x^2) = \left[x_1 + x_2^2\right]_{(5,0)} = 5,$

$$f_2(x^1) = \left[(x_1 - 5)^2 + x_2^2\right]_{(0,0)} = 25.$$

the corresponding fuzzy matrix is

$\mu_{f_i^j}$	x^1	x^2	f^u
f_1	0	0.1980	25.25
f_2	0.8333	0	30

Step 3. Substitute of pay- off table in relation (3) to obtain the corresponding compromise weights $\lambda_1 = 0.4545$ and $\lambda_2 = 0.54545$

Step 4. The new composite membership function is

$$\min \mu_{f^3}(x) = \min\left\{\frac{0.4545}{25.25}\left[x_1 + x_2^2\right] + \frac{0.54545}{30}\left[(x_1 - 5)^2 + x_2^2\right]\right\}$$

Subject to $x_1^2 + x_2^2 \le 25,$

$$x_1 \ge 0, \quad x_2 \ge 0.$$

The solution is

$x^3 = (4.504950495, 0), \; f_1(x^3) = 4.504950495,$

$f_2(x^3) = .2450740124$

Step 5. The set of all parameters which corresponds to this solution is defined by the stability set of the first kind in the following form:

$$S(x^3) = \{\lambda_1 - 0.990099010\,\lambda_2 + 9.009900990\,u_1 = 0,$$

$$\lambda_2 = 0, \; u_1 > 0\}$$

Step 6.

$$\mu_{f_1^3} = \frac{f_1(x^3) - f_1^I}{f_1^U - f_1^I} = \frac{4.504950495 - 0}{25.25 - 0} = 0.1784138810,$$

$$\mu_{f_2^3} = \frac{f_2(x^3) - f_2^I}{f_2^U - f_2^I} = \frac{0.2450740124 - 0}{30 - 0} = 0.00816913347.$$

Therefore, the new fuzzy matrix is

$\mu_{f_i^j}$	x^1	x^2	x^3	f^u
f_1	0	0.1980	0.1784138810	25.25
f_2	0.8333	0	0.00816913347	30

Step 7. Present the three solution to **DM** if he is certain that one of them is the best solution of the problem (not only preferred regarding the other two), stop. Else, ask DM whether he prefers one solution over the two solutions. Suppose that he would not, and his least preferred solution would be solution 2. This solution is then replaced by solution 3 return to Step 3.

The new pay-off table is

	f_1	f_2
f_1	4.504950495	5
f_2	0.2450740124	0

By using relation (3) we obtain the compromise weights

$\lambda_1 = 0.2487562190$ and $\lambda_2 = 0.7512437810$.

We note that these weights are out of the range of parameters which were defined in the above set $S(x^3)$ so we must have the next solution.

The new composite membership function is

$$\min \mu_{f^3}(x) = \min\left\{\frac{0.2487562190}{25.25}\left[x_1 + x_2^2\right] + \frac{0.7512437810}{30}\left[(x_1 - 5)^2 + x_2\right]\right\}$$

subject to $x_1^2 + x_2^2 \le 25,$

$$x_1 \geq 0, \quad x_2 \geq 0.$$

which solution is

$x^3 = (4.80329159, 0),\ f_1(x^3) = 4.803292,\ f_2(x^3) = 0.0386942.$

, the corresponding stability set of the first kind is

$$S(x^3) = \{\lambda_1\text{-}0.39356829\lambda_2 + 9.6064318\, u_1 = 0, \lambda_2 = 0, u_1 > 0\}$$

We have the corresponding membership function in the form

$\mu_{f_1^3} = 0.1902293698, \mu_{f_2^3} = 0.00128906658$.

Therefore the fuzzy matrix is

$\mu_{f_i^j}$	x^1	x^2	x^3	f^u
f_1	0.17841388	0.1980	01902293698	25.25
f_2	0.0081691334	0	0.001289806658	30

Suppose the DM would prefer the new solution over these solutions. Go to Step 8.

Step 8. Stop. The best compromise solution of this problem would be

$$\bar{x} = (4.80329158725595917, 0),$$
$$f = (4.803291587, 0.03869419974),$$
$$\mu_f = (0.1902293698, 0.001289806658).$$

7. Conclusion

An interactive stability compromise programming method , using a fuzzy approach and a pay-off table. In this method, no prior information is required from the **DM** and the compromise weights of the objective functions are determined from the pay-off table and fuzzy matrix. The method does not require significantly more data than pure nonlinear programming and the scale of multi-objective problem by using substituting the objective functions by the membership function and to obtain compromise weights by the grades of membership of the current vectors in each iteration in the "close ideal" fuzzy set.

The proposed algorithm programmed by using Maple program.

8. Reference

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9. Appendix

A maple program for solving multi-objective nonlinear programming (MONLP) problems and stability of this solution**.**

```
> restart:
with(Optimization):
with(Maplets[Elements]):
with(Groebner):
> maplo:=Maplet(["Enter The Type of The
Problem",
[Button("Minimize",Shutdown("Minimize")),
Button("Maximize",Shutdown("Maximize"))
]]):
d:=Maplets[Display](maplo):dm:=parse(d);
ma := Maplet([["Enter No of Vector Spaces",
TextField['TF']()],
[Button("ok",Shutdown(['TF']))]]):
n1:=Maplets[Display](ma):n:=parse(n1[1]);
q1:="a":i:=0:
while(q1="a") do
i:=i+1:
maplet := Maplet([["Enter an Objective
function ", TextField[TF1]()],
[Button['b']("ok",Shutdown( [TF1]))]]):
t[i]:=Maplets[Display](maplet);
maplet2 := Maplet([[Label("Enter Another
objective function?:")],
[Button['B1']("Ok",Shutdown("a"))],
[Button['B2']("No",Shutdown())]]):
q1:= Maplets[Display](maplet2):
end do:
> m:=i;
> for i from 1 to m do
```

```
f[i]:=parse(t[i][1]);
end do;
> q2:="a":i:=0:
while(q2="a")do
i:=i+1:
mapl1 := Maplet([["Enter Your Constraints",
TextField[TF1]()],
[Button['b']("ok",Shutdown( [TF1]))]]):
t1[i]:=Maplets[Display](mapl1);
mapl2 := Maplet([
[Label("Enter Another Constraint?: ")],
[Button['B1']("Ok",
Shutdown("a"))],[Button['B2']("No",
Shutdown())]]):
q2:= Maplets[Display](mapl2):
end do:
> k:=i;
> for i from 1 to k do
g[i]:=parse(t1[i][1]);
end do;
> for i from 1 to m do
for j from 1 to k do
> Q[i]:=Maximize(f[i], {g[j]},
assume=nonnegative); P[i]:=Minimize(f[i],
{g[j]}, assume=nonnegative);
> end do;Q[i];P[i];end do;
> for i from 1 to m do
for j from 2 to m+1 do
> R1[i,j]:=rhs(Q[i][2][j-1]);
> # Minimization.
> S1[i,j]:=rhs(P[i][2][j-1]);end do;
end do;
> nnn:=proc(R1,S1,Q,P,f,g,n,m,k,MU1)
local R,i,j,K,MUf,A,alpha,FN,MuF,mx,f1,
f2,f3,f4,f5,f6, maplet3:
global S,Mu,Lamda,Z,eq,ss,eq1,rr,su1,su2,lam,
alph,kk,UU,U:
for i from 1 to m do
> # Maximization.
R[i,1]:=Q[i][1];
> # Minimization.
> S[i,1]:=P[i][1];
for j from 2 to m+1 do
R[i,j]:=R1[i,j];
S[i,j]:=S1[i,j];
end do: end do:
> Z:=Matrix(1..m,1..m+1):
> for i from 1 to m do
for j from 1 to m do
f5[i]:=f[i]:f4[i]:=f[i]:
if i=j then
kk:=1:
while(kk<=m) do
f1[i]:=subs(x[kk]=S[i,kk+1],f5[i]):
f5[i]:=f1[i]:kk:=kk+1:
end do:
Z[i,i]:=f5[i]:
else
kk:=1:
while(kk<=m) do
f2[i]:=subs(x[kk]=S[j,kk+1],f4[i]):
f4[i]:=f2[i]:kk:=kk+1:
end do:
Z[i,j]:=f4[i]:
end if:
end do;
end do;
Z;
for i from 1 to m do
> MUf[i]:=(f[i]-S[i,1])/(R[i,1]-S[i,1]):
> end do;
Mu:=Matrix(1..m,1..m+2):
> for i from 1 to m do
> for j from 1 to m do
> Mu[i,j]:=(Z[i,j]-S[i,1])/(R[i,1]-S[i,1]);
 end do;
> Mu[i,m+1]:=R[i,1];
> end do: Mu;
> mx:=Array(1..m):
for i from 1 to m do
mx[i]:=Z[i,1];
for j from 1 to m do
if (mx[i]<Z[i,j]) then mx[i]:=Z[i,j]; end if;
end do;
end do;
mx;
Lamda:=Array(1..m):
A:=Array(1..m):for i from 1 to m do
> A[i]:=mx[i]-Z[i,i];
> end do;
> alpha:=ln(abs(add((A[i]/A[m]),i=1..m)))/
(A[m]-A[m-1]);
> for i from 1 to m do
> Lamda[i]:=exp(alpha*A[i])/
  add(exp(alpha*A[k1]),k1=1..m);
> end do;
> add(Lamda[i],i=1..m);
> FN:=add(Lamda[i]*MUf[i],i=1..m);
> for i from 1 to k do
MuF:=dm(FN, {g[k]}, assume=nonnegative);
end do:
> for j from 2 to n+1 do
S[m+1,j]:= rhs(MuF[2][j-1]); end do;
lam:=Vector(m,symbol=la):
```

```
UU:=Vector(k,symbol=U):
> for alph from 1 to n do
su1:=add(diff(f[i],x[alph])*lam[i],i=1..m);
su2:=add(diff(lhs(g[j]),x[alph])*UU[j],j=1..k);
> eq[alph]:=su1+su2;
end do:
> for i from 1 to m do
> j:=1:
f6[i]:=f[i]:
while(j<=m) do
f3[i]:=subs(x[j]=S[m+1,j+1],f6[i]);
f6[i]:=f3[i]:j:=j+1:
end do:
Z[i,m+1]:=f6[i];
> end do;Z;
> for i from 1 to m do
> Mu[i,m+2]:=Mu[i,m+1];
Mu[i,m+1]:=(Z[i,m+1]-S[i,1])/(R[i,1]-S[i,1]);
> end do:MU1:=print("Mu=",Mu);
end proc:
> nnn(R1,S1,Q,P,f,g,n,m,k,MU1);
RS:=Array(1..n):RS1:=Array(1..n):
for alph from 1 to n do
rr1:=eq[alph]:rr11:=eq[alph]: j:=1:
while(j<=m) do
rr:=subs([la[j]=Lamda[j],x[j]=S[m+1,j+1]],rr1):
r:=subs([x[j]=S[m+1,j+1]],rr11):
rr1:=rr: rr11:=r: j:=j+1:
end do:RS[alph]:=rr1;
RS1[alph]:=rr11;
end do:RS1;#RS;
syst:= [seq(RS[alph],alph=1..n)];
> var:=[seq(U[i],i=1..k)];
bs:=0;
printlevel :=4:
if IsProper(syst)=true then
B:=solve(syst,var);
for i from 1 to k do
if rhs(B[1][i])<0 then bs:=bs+1 end if:
end do:
if bs>=1 then "not stable" else "stable" end if;
else
"System is not stable" ;
 end if;
> maplet3 := Maplet([["agree these values?"],
[Button['q']("&OK", Shutdown("yes")),
Button['q1']("&No",Shutdown("No"))]]):
ss:=Maplets[Display](maplet3):
for i from 2 to n+1 do
print("x",m+1,"=",S[m+1,i]);end do;print("Pay-
Table=",Z);print("Lamda=", Lamda);
> while ss="No" do
mapl1:=Maplet([["Enter the no of x you want
to replace
with",TextField['TF']()],[Button("ok",Shutdown
(['TF']))]]):
d1:=Maplets[Display](mapl1):
d2:=parse(d1[1]):
unassign('ss');unassign('Mu');
unassign('Lamda');
unassign('Z');unassign('eq');unassign('MU1');
> for i from 1 to m do
> R1[d2,i+1]:=S[m+1,i+1];
> S1[d2,i+1]:=S[m+1,i+1];
for j from 1 to d2-1 do
S1[j,i+1]:=S[j,i+1];
end do;
for j from d2+1 to m do
S1[j,i+1]:=S[j,i+1];
end do;
end do;
> nnn(R1,S1,Q,P,f,g,n,m,k,MU1);
> maplet3 := Maplet([["agree these values?"],
[Button['q']("&OK", Shutdown("yes")),
Button['q1']("&No",Shutdown("No"))]]):
ss:=Maplets[Display](maplet3):
> end do:
RS:=Array(1..n):RS1:=Array(1..n):
for alph from 1 to n do
rr1:=eq[alph]:rr11:=eq[alph]:
j:=1:
while(j<=m) do
rr:=subs([la[j]=Lamda[j],x[j]=S[m+1,j+1]],rr1):
r:=subs([x[j]=S[m+1,j+1]],rr11):
rr1:=rr: rr11:=r: j:=j+1:
end do:
RS[alph]:=rr1;
RS1[alph]:=rr11;
end do:RS1;#RS;
syst:= [seq(RS[i],i=1..n)]:
> var:=[seq(U[i],i=1..k)]:
bs:=0;
> if IsProper(syst)=true then
B:=solve(syst,var);
for i from 1 to k do
if rhs(B[1][i])<0 then bs:=bs+1 end if:
end do:
if bs>=1 then "not stable" else "stable" end if;
else
"System is not stable" ;
 end if;
> if ss="yes" then
mapl4:=Maplet([["Which one you prefere,
x(",TextField['TF'](),")"],
```

```
[Button("ok",Shutdown(['TF']))]]):
dd:=Maplets[Display](mapl4):
d3:=parse(dd[1]):
> end if:
> for i from 1 to m do
> zz[i]:=subs({x[1]=S[d3,2],x[2]=S[d3,3]},f[i]);
> l:=d3;
> end do:
print("Mu=",Mu);
> for i from 1 to  m do
> print("x=",S[l,i+1]);end do:
for i from 1 to  m do
print("fmin=",zz[i]);
> end do;
for i from 1 to  m do
> print("Mu=",Mu[i,l]);
> end do;print("Pay-
Table=",Z);print("Lamda=",Lamda);
```

12/12/2010

Management of Recurrent Pterygia

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Abstract: PURPOSE: The objective of this study was to evaluate the postoperative outcomes of different surgical techniques with adjunctive therapy for the management of recurrent pterygia. **MATERIALS and METHODS:** Twenty eyes of twenty patients (7 females and 13 males, mean age 42.3 +/- 9.6 years) operated on for recurrent pterygia at the Research Institute of Ophthalmology, were recruited in this study. Patients were randomized into two groups: In group1, ten eyes of ten patients were done with conjunctival autograft and in group 2, ten eyes of ten patients were done with limbal conjunctival autografting. All eyes received intraoperative mitomycin C 0.01% for 3 minutes applied to the bare sclera at the time of the operation. The site of application of mitomycin C was thoroughly irrigated with balanced salt solution. All eyes were followed up every month for 12 months. **RESULTS:** After a mean postoperative follow up of 12 months, only one eye had a recurrence after 4 months in the limbal conjunctival autogrft group and there were two eye with recurrence after 2 and 4 month in the conjunctival autografting group ($p = 0.027$). No severe side effects appeared during the follow up period. **CONCLUSION:** This study confirms the efficacy of adjunctive therapy in improving the success rate after recurrent pterygium surgical excision. There was no difference between the two surgical procedures in the two groups, we also found no serious complications from using a low concentration (0.01%) of mitomycine C which was effective also in prevention of recurrences.

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Keywords: Management; Recurrent; Pterygia

1.Introduction:

Pterygium is a common external eye disease seen more frequently in tropical and subtropical areas due to exposure to ultraviolet sunlight. The main histopathological change in primary pterygium is elastodysplasia and elastodystrophy of subepithelial connective tissue,Austin et al, (1983)

Indications for surgical excision include impending or manifest visual loss due to involvement of the central cornea, irregular astigmatism, restriction of ocular motility, atypical appearance leading to concerns of squamous neoplasia,Hirst (2003)Surgical treatment of pterygium is directed at excision, prevention of recurrence, and restoration of ocular surface integrity. Also the main concern of simple excision of pterygium is the high recurrence rate as simple excision of the pterygium carries a high recurrence rate ranging from 24-89%, Jaros and Deliuse (1988). As an attempt to prevent recurrence adjunctive therapies are to be considered. These include antimetabolites as mitomycin C, radiotherapy, conjunctival or limbal conjunctival autograft and amniotic membrane graft ,Kenyon et al., (1985).

The addition of mytomycin C of various concentrations has been reported to be effective in preventing recurrence ,Lam et al.,(1998)

Unacceptable recurrence rates led to abandonment of the excision with bare sclera technique with widespread acceptance of conjunctival autografting, Troutbeck and Hirst (2001)

Limbal conjunctival autografting using stem cells is reported to be an effective alternative adjuvant to lower the recurrence rate of the pterygium ,Manning et al.,(1997). As the limbal epithelium acts as a junctional barrier to conjunctival overgrowth and pterygium is considered to represent a “local limbal deficiency” Tseng (1989). Also the inclusion of limbal epithelium in conjunctival graft would restore the barrier function of the limbus. Recent studies have reported the effectiveness of limbal conjuctival autograft transplantion (LCAG) in the prevention of pterygium recurrence, Rao et al, (1998); Gris et al.,(2000) ;Al Fayez (2002)

The aim of this study is to determine the recurrence rate after two surgical procedures of

pterygium excision and conjunctival autogrifting with or without limbal stem cell transplant.

2.MATERIALS and METHODS:

2.1.Materials:

2.1.1. Sample of the study:

Twenty eyes of twenty patients (7 females and 13 males, mean age 42.3 +/- 9.6 years) operated on for recurrent pterygia at the Research Institute of Ophthalmology, were recruited in this study.

Patients were randomized into two groups: in group 1, ten eyes of ten patients were done with conjunctival autograft and in group 2, ten eyes of ten patients were done with limbal conjunctival autografting.

2.1.2.Drugs:

Postoperative topical antibiotic and steroid therapy was used 4 times a day for 2 weeks and then 2 times a day for another 2 weeks for both groups.

2.2.Methods:

2.2.1. Surgery operations :

All surgeries were done under local anaesthesia. All eyes received intra-operative mitomycin C 0.01% for 3 minutes applied to the bare sclera at the time of the operation. The pterygium body was thoroughly removed and the pterygium head was undermined and removed by dissection or avulsion to reach the clear corneal lamelae. The site of application of mitomycin C was thoroughly irrigated with balanced salt solution while preparing the conjunctival autograft or the limbal conjunctival graft from the temporal conjunctiva by marking the area of conjunctiva to be incised and then injecting saline solution under the conjunctiva using an insulin syring to separate the conjunctiva from the tenon followed by excision of the graft. The conjunctival autograft was then sutured to the conjunctiva defects with the same orientation with 8/0 Vicryl interrupted sutures. In group 2 while harvestin stem cells at the limbus a 1 mm of the clear cornea has to be taken with conjunctiva and graft margins were secured to the recipient site while stem cells aspect was sutured to the limbus with 2 interrupted 10/0 silk sutrues. All eyes were followed up on the second day and one week postoperatively, then every month for another 12 months for the detection of early signs of recurrence and complications. Recurrence was defined as fibrovascular proliferation invading the cornea ≥ 1.5 mm; Lam et al.,(1989).

2.2.2. Statistical analysis:

Student T test was used to analyze recurrence rate and a P value less than 0.05 was considered significant.

3.Results:

After a mean postoperative follow up period of 12 months, only one eye had a recurrence after 4 months in the limbal conjunctival autograft group and there were two eye with recurrence after 2 and 4 month in eyes with conjunctival autografting group ($p = 0.027$) with no recurrences in the rest of the eyes in both groups (Figure 1,2,3). Minimal complications as superficial punctate keratitis, redness and irritations to the eyes were noticed in the first postoperative days due to the use of mitomycin C which were resolved spontaneously. No severe side effects appeared during the follow up period.

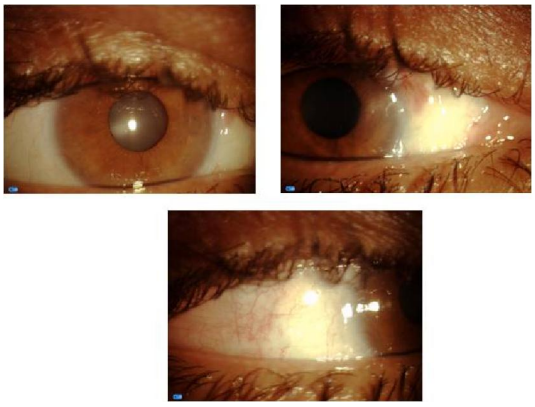

Fig. (1) One year post pterygium excision with conj. autograft

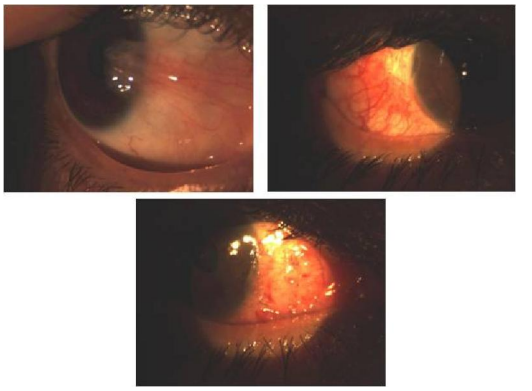

Fig. (2) Pre and post pterygium excision with limbal conj. autograft

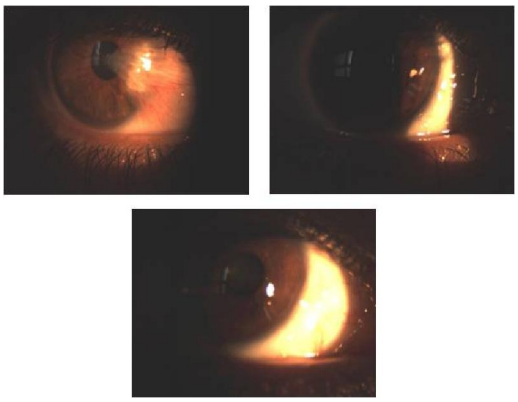

Fig. (3) Pre and post pterygium excision with conj autograft

4. DISCUSSION:

As regards the high recurrence rate after pteryguim excision, adjunctive therapy is very essential in order to decrease the rate of recurrence.

Cases recruited in this study were recurrent cases that needed adjunctive therapy to decrease the rate of recurrences.

Recent studies have reported the effectiveness of limbal conjuctival autograft transplantion (LCAG) in the prevention of pterygium recurrence due to the theory that limbal stem cell deficiency lead to the progression of the pterygium limbal autograft with limbal epithelium in conjunctival graft would restore the barrier function of the limbus. Rao et al, (1998); Gris et al.,(2000) ;Al Fayez (2002).Limbal conjunctival autograft transplantation with recurrent pterygium was found to be a successful method to prevent recurrences in patients under 40 years of age with a recurrence rate of 13.3% after a mean follow up period of 10 months (Ranging 3-18 months) compared to 50% recurrence rate without limbal transplantation (control group) ,Guler et al, (2010)

In an attempt to decrease the recurrence rate, surgical time and post operative pain, the use of fibrin adhesive in primary pteryguim surgery with conjuctival autograft was found to reduce the recurrence rate to 4.41% in comparison to 15.9% in the suture group, it also reduces the surgical time and the post operative pain when compared with suture groups ,Ratnalingam et al., (2010).

A recurrence rate of 4.75% with no signs of complications was found after limbal stem cells and conjunctival autograft transplantation ,Soliman and Bhatia (2009).

It was also proven that the procedure is an effective surgical technique in preventing pteryguim reccurence and it can also help in improving the best corrected visual acuity,Abdalla (2009).

Recurrent pteryguim exhibits a more aggressive fibrovascular growth pattern leading to corneal and cojunctival scarring and limbal stem cell deficiency. Proper excision of pathological tissue with amniotic membrane transplantation and mitomycine C represents an alternative surgical method with good final outcome ,Jirásková and Rozsíval (2008).

In managing chronically recurring pteryguim combined surgical procedure of pteryguim excision with amniotic membrane transplantation, conjuctival limbal autograft and mitomycine C application seems to be beneficial,Sangwan et al., (2003).

If mitomycine C is contraindicated, inferior limbal conjuctival autograft appears to be safe and effective option in the management of recurrent pteryguim,Wong et al.,(2000).

Mitomycine C is a potent cytotoxic agent that inhibits DNA synthesis resulting in all cycle arrest in the S phase.We used it in a low dose in a concentration of (0.01%) in an attempt to decrease the incidence of ocular surface complication.

Intraoperative mitiomycine C has been shown to be highly effective in improving the success rate after recurrent pteryguim surgical excision after a mean follow up period of 34-55 months a recurrence rate of 12.5% in the mitomycine group and 35.6% in the control group. The 24 and 48 month success rate were 89% and 83% in mitomycine treated groups and 66% and 63% in the other group respectively. No severe side effects during the follow up period. Superfacial puctate keratits appeared in the early postoperative period in only 25.5% of the cases, Mastropasqua et al., (1996).

Although the use of mitomycine C decrease the reccurence rate of pterguim, the ideal application method and the dose still remain controversial. A concentration of 0.02% mitomycine C is an effective treatment for the prevention of recurrent pteryguim,Hosal and Gursel (2000).

It was also found that amniotic membrane closure and conjuctival autograft combined with mitomycine C are effective to prevent recurrence in the treatment of recurrent pteryguim, Katircioğluet al.,(2007).

Some authors found that conjunctival limbal autografting and amniotic membrane methods were more effective and safer than intraoperative mitomycine C ,Keklikci et al., (2008).

In a comparison between limbal conjuctival autograft transplantation versus mitomycine C with conjuctival flap in the treatment of recurrent pertyguim surgery, both techniques showed similar reccurence rates ,Mutlu et al.,(1999).

Also, some authors found that in treating recurrent pteryguim, simple excision and low-dose mitomycine C followed by limbal conjuctival autografted is a safe and effective way ,Nabawiet et al.,(2003).

Most of authors agreed about the treatment of recurrent pteryguim as patients requires a more radical excision with a large autograft and the use of adjuncts such as mitomycine C ,Massaoutis et al., (2007).

Also some surgeons agreed that amniotic membrane graft was as effective as conjuctival autograft and mitomycine C in preventing pterguim reccurence ,Ma et al.,(2000).

Thus, it can be concluded that combined intraoperative mitomycine C, amniotic membrane graft and limbal conjuctival autograft are successful approaches for treating multireccurent pterygia with severe symblepharon to restore the ocular surface integrity and prevent recurrence, Yao et al., (2006).

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Prevalence, Risk Assessment and Impacts of Eye Diseases among School Children in Cairo, Egypt

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Abstract: Introduction: Eye diseases represent an important public health problem in childhood. Objectives: The aim of this study was to define the prevalence of different types of eye diseases, to assess risk of these diseases, and to determine the disease impacts on scholastic achievement of school students in Cairo, Egypt. Research design: A cross-section, analytical study design was chosen to perform this study. Research setting: The study was conducted in Al-Marg region, east district of Cairo. Four, randomly selected, schools were the field of the present study in this region. These schools were two primary schools (one public and one private) and two preparatory schools (one public and one private). Subjects and methods: The total number of students was 2160. All the students were examined clinically; for each case with eye disease a control case was chosen. The cases and controls were interviewed. Results: The study showed that 28.2% of the students have eye diseases. The most common eye diseases were trachoma (9.3%), errors of refraction (7.1%) and allergic conjunctivitis (6.3%). All eye diseases were more common in public schools. The most important significant socioeconomic and health care behavioral risk factors for eye diseases were the low level of parental occupation (OR=4.79), no early consultation for eye diseases (OR=3.13) and never received eye examination (OR= 2.68). Also, the most important significant personal characteristic risk factors were previous eye diseases (OR=3.35), positive consanguinity of the parents (OR=2.67), sibling(s) with eye diseases (OR=2.19), last birth order child (OR=1.90) and male sex (OR=1.56). Further, age and/or sex were significant risk factors for specific eye diseases; trachoma, errors of refraction, allergic conjunctivitis and muco-purulent conjunctivitis. Also, 37.7% of the students with eye diseases had significant school absenteeism 3-4 days/month (P=0.01) and 21.8% of them had significant results of the first term exam <50.0% (P=0.00). Conclusions: Eye diseases are prevalent among school students, especially in public schools in Cairo, Egypt. Many of the risk factors of eye diseases can be manipulated. So, these diseases and its negative impacts can be prevented. Recommendation: Improving students' and environment's hygiene, health education, regular eye screening and treatment of students as regard eye diseases in Egypt are an important essentiality. Also, eye health component of school health services should be integrated in school health program, and this should be integrated in medical and nursing curriculums. Lastly, further studies on large numbers of students in different rural and urban areas in Egypt are recommended.

[**Essam A. El-Moselhy; Hosam S. Abo-Seif; Eman S. Abd Allah and Ahmed A. Ghandor. Prevalence, Risk Assessment and Impacts of Eye Diseases among School Children in Cairo, Egypt**. Journal of American Science 2011;7(1):235-246]. (ISSN: 1545-1003). http://www.jofamericanscience.org.

Key words: Eye Diseases, School Children, Impacts

1. Introduction:

School children are considered one of the most important sectors of population due to their continuous growth and development at all levels. They are a vulnerable group and great attention should be paid for them (Abdel-Wahab and Mahmoud, 1987). So, coordinated school health programs in conjunction with community efforts can prevent many health problems among students and help them to establish lifelong safety skills (Allensworth et al., 1997 and El-Moselhy et al., 2005a).

Vision is an important requirement for learning and communication. Further, optimal vision is essential for learning, health and educational needs (Adegbehingbe et al., 2005). So, eye diseases are a public health problem (Alakija, 1995). But, some eye care and public health professionals have argued that every child should receive a comprehensive examination by an optometrist or ophthalmologist at school entrance. While, others maintain that vision screening is a cost-effective method for identifying those who would benefit from eye exams. These competing recommendations for how best to identify children with vision problems are prompting new research on the costs and benefits of various strategies, including an examination of the impact of

untreated vision problems on school performance (Ferebee, 2007).

Approximately 1.4 million children in the world are blind; 75.0% of them live in developing countries. For every blind child, three children have serious vision impairment and 13 need eyeglasses (The USAID Child Blindness Program, 2007). Most of the blindness or serious eye problems can be prevented if detected at an early stage. So, the screening of eye health of the school children is important procedure; it will reveal most of the problems and thus prevent many visual defects (Al-Nasser et al., 1989; Donaldson, 2002 and Adegbehingbe et al., 2005). To ensure early detection of visual defects students should be examined early in the primary school (Badr and Qureshi, 1981). Primary care clinicians can play a vital role in preserving vision by ensuring that patients undergo periodic evaluations by eye care professionals and receive needed eye care (Rowe et al., 2004).

Prevalence of eye diseases differ in different communities according to many factors, which include social and environmental characters of the community, health habits of the community, personnel hygiene and technical methods used in diagnosis of eye diseases (Al-Nasser et al., 1989 and Abdou et al., 2007).

At present, trachoma remains the most important infectious cause of blindness in the world (Resnikoff et al., 2004). Repeated infection with the ocular strains of *Chlamydia trachomatis* can bring about scarring of the conjunctiva, resulting in a cascade of entropion, inward-turned eyelashes, and eventually blindness due to corneal opacity (Mariotti, 2004). In Egypt, the rate of inflammatory eye diseases among rural population was unchanged since 1920s (Courtright et al., 1989). Trachoma infection among primary school children in Upper Egypt was found to be 64.1% (Rashwan and Mohamed, 1992). The prevalence of trachoma was about 71.0% in KSA (Badr, 1982a&b). But it starts to decrease to about 22.0% in the population (Faran and Tabbara, 1987).

Eye health problems among school children in developing countries have increased over time (Al-Nasser et al., 1989). It is estimated that 7.0% of new schools' entrants have vision defects. About 6.0% of boys are likely to be color blind and 1.8% of the children have squint. Also, 3.3% of school children had visual loss due to lack of eye care, 2.3% of boys had squint and 1.8% had refractive error (Badr, 1982a&b). Further, 12.0% of school children had refractive error, 2.8% had squint and 0.7% had color blindness error (Al-Nasser et al., 1989). Also, the most common refractive error among students of a female preparatory school in Jeddah, KSA was found to be myopia, it represents 12.6% of the studied group (Salem, 1999).

Prevalence of corneal scarring among children in Assiut governorate was 2.1% (Farahat et al., 1986). Recently, its prevalence among school children aged 6-18 years in Sohag governorate was 1.8% (Mohamed, 1998).

Allergic conjunctivitis affects between 10.0-15.0% of the total United Kingdom population (McGill et al., 1998). While, it was found among 8.7% of the studied group of females preparatory schools in KSA (Salem, 1999).

Lost opportunities associated with blindness and visual impairment lead to emotional stress and economic hardship. Blind children and those with limited sight experience social isolation, low self-esteem, lack of independence, and lost educational and economic opportunities. Lack of eye care can have a severe economic impact by perpetuating poverty or pushing a family into poverty (The USAID Child Blindness Program, 2007).

Eye health service component of school health services has prime importance and is responsible for early detection of refractory errors, correction of squint and amblyopia, detection and treatment of eye infections such as trachoma (Al-Nasser et al., 1989).

Study objectives:

A- Ultimate objective:

Improving quality of eye health of the school children in Egypt.

B- Immediate objectives:

1- To determine the prevalence of eye diseases among school children in Cairo, Egypt.
2- To assess the sociodemographic, environmental and health care behavior risk factors for eye diseases among school children in Cairo, Egypt.
3- To determine the impacts of eye diseases on the school absenteeism and scholastic achievement of the school children in Cairo, Egypt.

2. Subjects and Methods:

A- Technical Design

I- Research Questions:

What are the most common prevalent eye diseases among school children in Cairo, Egypt? Is there sociodemographic, environmental and health care behavior factors effects on prevalence of eye diseases among these school children? Is there effects of eye diseases on school absenteeism and scholastic achievement of school children in Cairo, Egypt?

II- Research Design:

A cross-section, analytical study design was chosen to investigate the current research problem.

III- Research Setting:

This study was conducted in Al-Marg region, east district of Cairo, Egypt. One primary and one preparatory public school were chosen randomly in this region. Also, one primary and one preparatory private school were chosen randomly in the same region. These schools were the field of the present study.

VI- Research Sample:

In each school, three classes were chosen randomly in each educational class level. So, these classes were fifty four; all students of these classes were recruited and examined. The total number of students was 2160; 1210 in public schools and 950 in private schools. The students aged from 6-16 years. For each case with eye disease a control case was chosen from the students' class list, the name after the diseased case. The cases and controls were interviewed, in case of young and/or non-cooperative student one of his/her parents was interviewed.

VI- Research Tools and Methods:

1- Diagnosis of childhood eye diseases: All students included in the study had undergone full physical examinations to detect those with eye diseases. The suspicious cases were invited to the investigator's private clinic for further examinations. All students with eye diseases were managed freely. Clinical examination included:

1-1- General eye condition was observed in good illumination.

1-2- Visual acuity was tested by Landolt's broken rings chart. Students were seated at six meters distance from the chart in good illumination. The student was asked to refer (indicate) to the direction of the opening of the ring while he/she covering one eye and so the other eye in turn.

1-3- Stages of trachoma were diagnosed according to Loewenthal and Pe're (1990).

1-4- Tonometry was done by palpation and by tonometer in the suspicious cases.

1-5- Squint cover and uncover test was done using a piece of carton while the student focusing on near and far objects. Hirschberg method was done where the light source was held at a distance of fifty cm from the student's head to observe the angle of deviation according to the corneal light reflection.

1-6- Color vision was tested by Ishhara chart.

1-7- Diffuse and focal illumination & direct and indirect ophthalmoscopy were, also, done.

2- Interview questionnaire: It was used to collect data relevant to topic of the study. Also, one of the student's parents was submitted to an interview if needed.

3- Scholastic achievement:

It was determined according to results of the first term exam; very good/excellent (>80.0%), passed/good (50.0-80.0%) and failed (<50.0%).

B- Operational Design

I- Preparatory Phase (pilot study): A pilot study was done on 100 students to test the feasibility of the study at the study sites and to measure the time and resources needed for the field work.

II- Ethical Consideration:

A verbal agreement, consent, from all the students' parents to participate in the research was taken after full explanation of the aim of the research. The participants' parents were assured that the researcher's will investigate and treat all positive cases and the parents will be informed.

III- Practical Phase:

This phase took about 4 months. The students were examined and the data were collected, in the second term, through field visits.

IV- Statistical Design:

Odds ratio (OR) with 95% confidence interval (CI) or exact confidence limits (ECL) was used to assess the risk. Also, Yates corrected Chi-square (χ^2) and Fisher exact (FE) were used as tests of significance. The significance level for χ^2 and Fisher exact was accepted if the P-value <0.05.

3. Results:

The overall percent of eye diseases among the studied school children (table 1) was 28.8%. In details; the percent of trachoma was 9.3% (active cases were 58.2% and inactive were 41.8%). As regard errors of refraction, 7.1% of our students had errors of refraction (corrected cases were 56.2% and uncorrected were 43.8%). Regarding allergic conjunctivitis, 6.3% of our students had allergic conjunctivitis. Also, 1.9% of the students had phylecten. At the same time, 1.6% of the students had squint. As regard color blindness, 0.9% of the students had color blindness. Regarding muco-purulent conjunctivitis (MPC), 0.8% of the students had MPC. Lastly, corneal scarring was found among 0.4% of the students.

Distribution of cases with eye diseases of students in both public and private schools is presented in table (2), the total number of eye

diseases among the studied public schools children was 441 (36.5%) compared with 181 (19.1%) among the studied private schools children with a statistically significant difference (P<0.001). At the same time, the total number of students with eye diseases among the studied public schools children was 432 (35.7%) compared with 178 (18.7%) among the studied private schools children with a statistically significant difference (P<0.001). In details; the percent of trachoma among the studied public schools children was 13.6% (active cases were 56.7% and inactive were 43.3%) compared with 3.9% (active cases were 64.9% and inactive were 35.1%) among the studied private schools children. These differences are statistically significant (P<0.001). At the same time, muco-purulent conjunctivitis was found among 1.2% of the studied public schools children compared with 0.3% among the studied private schools children. This differences are statistically significant (P=0.04). As regard errors of refraction, 7.2% of the students in public schools had errors of refraction (corrected cases were 3.4% and uncorrected were 3.8%) compared with 6.9% of the students in private schools (corrected cases were 4.7% and uncorrected were 2.2%). The differences as regard prevalence in general and the corrected are statistically insignificant (P=0.89 and P=014, respectively). While, the difference between prevalence the uncorrected is statistically significant (P=0.05). Regarding allergic conjunctivitis, 7.5% of our students in public schools had allergic conjunctivitis compared with 4.8% of the students in private schools. The difference is statistically significant (P=0.01). At the same time, 2.6% of our students in public schools had phylecten compared with 1.1% of the students in private schools. The difference is statistically significant (P=0.02). Also, 2.2% of our students in public schools had squint compared with 0.6% of the students in private schools. The difference is statistically significant (P=0.01). Lastly; color blindness (0.9%), corneal scarring (0.6%), epiphora (0.4%) and ptosis (0.3%) were found among our students in public schools compared with 0.8%, 0.2%, 0.1% and 0.1% of the students in private schools. The differences are statistically insignificant.

As regard distribution of cases with eye diseases and control group of students according to complaint (table 3), we noticed that all symptoms of eye diseases were not present among the controls except headache; 15.7% and 11.0% among the cases and controls respectively. The difference is statistically significant (P=0.02).

As respect socioeconomic risk factors (table 4), the low level of parental education (illiterate or primary), low level of parental occupation (unskilled labor) and low social level were significant risk factors for eye diseases (OR=2.71, 95% CI: 2.18-3.45; OR=4.79, 95% CI: 3.43-6.70 and OR=3.08, 95% CI: 2.36-4.01, respectively). On the other hand, the high level of parental education (university), high level of parental occupation (professional) and high social level were significant protective factors for eye diseases (OR=0.37, 95% CI: 0.29-0.47; OR=0.35, 95% CI: 0.51-0.44 and OR=0.37, 95% CI: 0.29-0.47, respectively).

Regarding health care behavior risk factors (table 5); the poor eye and environmental hygiene are significant risk factors for eye diseases (OR=1.37, 95% CI: 1.06-1.75 and OR=1.41, 95% CI: 1.11-1.80, respectively). Lastly, no early consultation for eye diseases and incompliance with therapy were risk factors eye diseases (OR=3.13, 95% CI: 2.46-4.00 and OR=1.29, 95% CI: 1.02-1.63, respectively).

In respect of personal characteristics risk factors (table 6), yonger age group 6-8 years was insignificant protective factor (OR=0.82, 95% CI: 0.63-1.06). On the other hand, older age group 12-16 years was insignificant risk factor (OR=1.13, 95% CI: 0.89-1.43). At the same time, male sex was significant risk factor (OR=1.56, 95% CI: 1.23-1.98).Also, first and last birth order child were significant protective with risk factors, respectively (OR=0.45, 95% CI: 0.35-0.58 and OR=1.90, 95% CI: 1.22-2.45, respectively). In addition, previous eye diseases and sibling(s) with eye diseases represented significant risk factors (OR=3.35, 95% CI: 2.63-4.28 and OR=2.19, 95% CI: 1.67-2.87, respectively). Lastly, positive consanguinity among parents represented a significant risk factor for eye diseases among their offspring's (OR=2.67, 95% CI: 1.03-5.55).

As regard the impacts of eye diseases (table 7); 41.2%, 37.7%, 21.1% and 52.3%, 30.3%, 17.4% of the students with eye diseases and controls, respectively had school absenteeism 0-2, 3-4 and ≥5 days/month, respectively. The difference was statistically significant regarding school absenteeism 3-4 days/month (P=0.01). As respect scholastic achievement; 21.8%, 34.6%, 43.6% and 12.6%, 41.2%, 46.2% of the students with eye diseases and controls, respectively had results of the first term exam <50.0%, 50.0-80.0% and >80.0%, respectively. The differences were statistically significant respecting to results of the first term exam <50.0% (P=0.00) and 50.0-80.0% (P=0.02).

As regard distribution of different cases with eye diseases and control group of students according to age and sex (table 8); younger age group 6-8 years and male sex were significant risk factors for trachoma (OR=2.12, 95% CI: 1.51-2.99 and OR= 1.39, 95% CI: 0.99-1.95, respectively). While, older

age group 12-16 years and female sex were significant risk factors for errors of refraction (OR=2.51, 95% CI: 1.72-3.67 and OR=1.47, 95% CI: 1.02-2.14, respectively). Also, big age group 12-16 years was significant risk factor for allergic conjunctivitis (OR=2.28, 95% CI: 1.53-3.38). On the other hand, male sex was insignificant risk factor (OR=1.20, 95% CI: 0.81-1.77). At the same time, small and middle age groups 6-8 and 9-11 years, respectively were insignificant risk factors for phylecten. Also, we cleared that male sex was insignificant risk factor for squint.

Table (1): Distribution of eye diseases among the studied school children.

Eye diseases	No. (n=2160)	Percent
Trachoma:	201	9.3
Active	117	58.2
Inactive	84	41.8
Errors of refraction:	153	7.1
Corrected	86	56.2
Un corrected	67	43.8
Allergic conjunctivitis	137	6.3
Phylecten	41	1.9
Squint	34	1.6
Color blindness	19	0.9
Muco-purulent conjunctivitis (MPC)	18	0.8
Corneal scarring	9	0.4
Epiphora	6	0.3
Ptosis	4	0.2
Total number of eye diseases	622	28.8
Total number of students with eye diseases	610	28.2

Table (2): Distribution of cases with eye diseases of students in both public and privateschools.

Eye diseases	Public schools (n=1210)		Private schools (n=950)		χ^2 FE	*P*-value
	No.	%	No.	%		
Trachoma:	164	13.6	37	3.9	56.10	0.000
Active	93	56.7	24	64.9	23.66	0.000
Inactive	71	43.3	13	35.1	27.63	0.000
Errors of refraction:	87	7.2	66	6.9	0.02	0.893
Corrected	41	47.1	45	68.2	2.19	0.138
Un corrected	46	52.9	21	31.8	3.97	0.046
Allergic conjunctivitis	91	7.5	46	4.8	5.98	0.014
Phylecten	31	2.6	10	1.1	5.73	0.016
Squint	27	2.2	7	0.6	6.74	0.009
Color blindness	11	0.9	8	0.8	0.00	0.946
Muco-purulent conjunctivitis	15	1.2	3	0.3	4.44	0.035
Corneal scarring	7	0.6	2	0.2	FE	0.313
Epiphora	5	0.4	1	0.1	FE	0.238
Ptosis	3	0.3	1	0.1	FE	0.635
Total number of eye diseases	441	36.5	181	19.1	77.68	0.000
Total number of students	432	35.7	178	18.7	74.75	0.000

Table (3): Distribution of cases with eye diseases and control group of students according to their complaint.

Complaint	Cases (n=610)		Controls (n=610)		χ^2	P-value
	No.	%	No.	%		
Eye itching	**213**	**34.9**	**0**	**0.0**	255.64	0.000
Headache	**96**	**15.7**	**67**	**11.0**	5.55	0.018
Sight defect	**74**	**12.1**	**0**	**0.0**	76.66	0.000
Eye disfigurement	**52**	**8.5**	**0**	**0.0**	52.25	0.000
Eye discharge	**18**	**3.0**	**0**	**0.0**	16.30	0.000

Table (4): Distribution of cases with eye diseases and control group of students according to their socioeconomic risk factors.

Socioeconomic risk factors	Cases (n=610)		Controls (n=610)		OR (95% CI)
	No.	%	No.	%	
Parental educational level:					
Illiterate & primary	318	52.1	175	28.7	2.71 (2.12-3.45)
Preparatory & secondary	102	16.7	99	16.2	1.04 (0.76-1.42)
University	190	31.2	336	55.1	0.37 (0.29-0.47)
Parental occupational level:					
Unskilled labor	199	32.6	56	9.2	4.79 (3.43-6.70)
Semi-skilled & skilled labor	222	36.4	211	34.6	1.08 (0.85-1.38)
Professional	189	31.0	343	56.2	0.35 (0.51-0.44)
Social level:					
Low	259	42.5	118	19.3	3.08 (2.36-4.01)
Middle	162	26.5	158	25.9	1.03 (0.80-1.35)
High	189	31.0	334	54.8	0.37 (0.29-0.47)

Table (5): Distribution of cases with eye diseases and control group of students according to health care behavior risk factors.

Health care behavior risk factors	Cases (n=610)		Controls (n=610)		*OR (95% CI)*
	No.	%	No.	%	
Eye hygiene:					
Good	396	64.9	437	71.6	0.73 (0.57-0.94)
Poor	214	35.1	173	28.4	1.37 (1.06-1.75)
Environmental hygiene:	362	59.3	411	67.4	0.71 (0.56-0.90)
Good	248	40.7	199	32.6	1.41 (1.11-1.80)
Poor					
Have ever received eye examination?	373	61.1	493	80.8	0.37 (0.29-0.49)
Yes	237	38.9	117	19.2	2.68 (2.05-3.50)
No					
Early consultation for eye diseases:	258	42.3	425	69.7	0.32 (0.25-0.41)
Yes	352	57.7	185	30.3	3.13 (2.46-4.00)
No					
Compliance with therapy:	226	37.0	263	43.1	0.78 (0.61-0.98)
Yes	384	63.0	347	56.9	1.29 (1.02-1.63)
No					

Table (6): Distribution of cases with eye diseases and control group of students according to personal characteristics risk factors.

Characteristics risk factors	Cases (n=610)		Controls (n=610)		OR (95% CI)
	No.	%	No.	%	
Age:					
5-8	152	24.9	176	28.8	0.82 (0.63-1.06)
9-11	203	33.3	197	32.3	1.05 (0.82-1.34)
12-16	255	41.8	237	38.9	1.13 (0.89-1.43)
Sex:					
Male	399	65.4	334	54.8	1.56 (1.23-1.98)
Female	211	34.6	276	45.2	0.64 (0.50-0.81)
Birth order:					
First	166	27.2	276	45.2	0.45 (0.35-0.58)
In the middle	199	32.6	175	28.7	1.20 (0.94-1.55)
Last	245	40.2	159	26.1	1.90 (1.22-2.45)
Previous eye diseases:					
Yes	355	58.1	179	29.3	3.35 (2.63-4.28)
No	255	41.8	431	70.7	0.30 (0.23-0.38)
Sibling(s) with eye diseases:	210	34.4	118	19.3	2.19 (1.67-2.87)
Yes	400	65.6	492	80.7	0.46 (0.35-0.60)
No					
Positive consanguinity of the parents:	31	5.1	12	2.0	2.67 (1.30-5.55)
Yes	579	94.9	598	98.0	0.37 (0.18-0.77)
No					

4. Discussion:

Blindness and visual impairment persist despite significant reductions in blindness through public health measures. Poverty, lack of primary health care and eye services, and unavoidable causes are major factors contributing to blindness. Injuries, genetic conditions, degenerative disorders, harmful eye treatments, and preventable infectious and non-communicable diseases; rarely found in industrialized countries; can cause blindness and visual impairment (The USAID Child Blindness Program, 2007). Also, as optimal vision is essential for health, learning and educational needs (Adegbehingbe et al., 2005); so, eye diseases especially that affecting vision are an important public health problem (Alakija, 1995).

Trachoma infection among primary school children in Kena Governorate, Egypt was found to be 64.1% (active cases were 78.8% and inactive were 21.2%) (Rashwan and Mohamed, 1992). Also, the prevalence of trachoma was 43.0% and of infection was 21.0% (Abdou et al., 2007). This result is so higher than ours. There are many factors that influencing the intensity of trachoma infection in children. Some of these factors may be cultural or site specific, while other may be environmental (Potter, 1991). On the other hand, our result is similar to the prevalence of trachoma (about 10.0%) among rural school children in KSA (Cross, 1985 and Faran & Tabbra, 1987). At the same time, errors of refraction are of the most common eye problems (Reddy et al., 2008). Our figure (7.1%) is similar to that estimated in primary schools in KSA; 7.0% of new schools' entrants have vision defects. Further, 12.0% of the eleven year old with normal vision show a defect at 16 years (Badr and Qureshi, 1981). Moreover, 18.7% of school children in Thailand had refractive errors (Nanthavisit et al., 2008). Also, 3.3% of school children in KSA had visual loss due to lack of eye care (Badr, 1982a&b). In addition, 12.0% of school children in KSA had refractive error; 38.3% of them were detected during research examination (Al-Nasser et al., 1989). In the Baltimore Vision-Screening Project the estimated prevalence of visual morbidity was found to be 8.2% for refractive errors (Presian and Norak, 1996). In addition, 3.1% of school students in Nigeria had refractive errors. None of them had an eye examination in the past (Adegbehingbe et al., 2005). At the same time, the most common refractive error among students of preparatory school in Jeddah, KSA was found to be myopia; it represents 12.6% of the studied group (Salem, 1999). On the other hand, myopia was found

among 1.3% of rural residents aged up to 20 years (Khallaf and Khalifa, 2004). Also, in the Indian study, 5.1% of the children in schools had a visual acuity of <6/12 in the better eye while 12.5% had a visual acuity of 6/9 or worse in either eye (Kalikivayi et al., 1997). This small figure could be explained; only one type of errors of refraction and their group contain infants and young children who can't indicate their own visual acuity. Also, in the US prevalence of reported visual impairment and blindness among children aged 6-17 years was 3.3% (CDC, 2005). Regarding allergic conjunctivitis, it was found among 8.1% of a studied group of rural residents aged up to 20 years (Khallaf and Khalifa, 2004). Also, it was found among 8.7% of a studied group of preparatory schools in KSA (Salem, 1999). On the other hand, it is less than the prevalence of allergic rhino-conjunctivitis (15.3%) among school children in Cairo (Georgy et al., 2006). Also, it is less than the 11.0% of the Nigerian school students; only 12.5% of them had visited an eye specialist at one time or the other (Adegbehingbe et al., 2005). At the same time, 1.9% of our students had phylecten. This figure is close to Khallaf and Khalifa (2004), who reported 2.1%. Strabismus (squint) is misalignment of visual axis of the eye in such a way that an object in the space is not visualized simultaneously by focus of each eye (Cross et al., 1985). It is suggested that early detection and correction of squint will prevent irreversible amblyopia (Al-Nasser et al., 1989). In primary schools in KSA the prevalence of squint among students was 1.8% (Badr and Qureshi, 1981), 2.3% (Badr, 1982a&b) and 2.8% (Al-Nasser et al., 1989). In the Baltimore, the US it is estimated that prevalence of squint was found to be 3.1% for strabismus (Presian and Norak, 1996). While, in Nigeria the prevalence of squint among students was 1.3% (Adegbehingbe et al., 2005). As regard color blindness, it is one of the congenital visual defects. At present time it is beyond correction (Al-Nasser et al., 1989). In primary schools in KSA it is estimated that 6.0% of students are likely to be color blind (Badr and Qureshi, 1981). Also, 0.7% of school children in KSA had color blindness (Al-Nasser et al., 1989). Regarding MPC, our figure is less than that (2.1%) estimated among rural residents aged up to 20 years (Khallaf and Khalifa, 2004). Our small figure could be explained; more hygienic environment, higher socioeconomic status and our group didn't contain infants and young children younger than six years who can't take care of their own cleanliness. Lastly, our figure regarding corneal scarring (0.4%) is in accordance with Adegbehingbe et al. (2005) (0.4%) and much smaller than figures reported by Farahat et al. (1986) and Mohamed (1998); 2.1% and 1.8%, respectively. This might be attributed to the recent advancement in health care of the eye.

The prevalence of eye infections in private school children is less than that in public schools (Shrestha et al., 2009). Most of the patients with eye diseases in Nigeria were from the lower socioeconomic class (Ajaiyeoba and Scott, 2002). This relation could be linked in certain eye diseases particularly of infectious origin to ignorance, poverty and bad environmental hygiene (Ajaiyeoba et al., 1996 and Hesselbarth, 2005). In the US, Hispanic children had significantly higher prevalence of reported visual impairment and blindness (3.6%) than non-Hispanic white children (2.3%). Also, children whose families were below the federal poverty level were nearly twice as likely to be visually impaired as children from families whose income was ≥200.0% of the poverty level. Moreover, children from families with incomes equal or more than twice of the federal poverty level were more likely to see an eye-care provider during the preceding year than children from families with incomes below poverty level (22.7% vs. 17.0%) (CDC, 2005). In India, the home of largest number of blind children in the world, among the rural population of the economically backward states childhood blindness is alarmingly high (Jain et al., 2005). Also, the two inflammatory eye diseases; trachoma and MPC are more common in unhygienic environment and among low socioeconomic standard population (Potter, 1991; Rashwan and Mohamed, 1992; Ajaiyeoba et al., 1996; Ajaiyeoba & Scott, 2002 and Hesselbarth, 2005). So the differences in their percent in public and private schools could be explained; more number of students in public class rooms compared with private schools, low hygienic environment and low socioeconomic status.

Also, we noticed that all symptoms of eye diseases were not present among the controls except headache. This result is consistent with Al-Nasser et al. (1989), they showed that headache was significantly more present among their students with refractory errors (P=0.0001). As refractory errors have found to be associated with headache, so headache is present more among our studied group. On the other hand, headache is a general symptom for many childhood diseases such as anemia, so its presence in controls was expected. In Egypt, about 50.0% of children are suffering from headache and anemia (UNICEF, 2000 and El-Masry et al., 2007).

As regard socioeconomic risk factors, poverty is a major factor contributing to blindness and visual impairments (The USAID Child Blindness Program, 2007). The majority (73.0%) of patients in Nigeria were from the lower socioeconomic class (Ajaiyeoba and Scott, 2002). This relation could be

linked in certain eye diseases particularly of nutritional and infectious origin to ignorance, poverty and dirty environment (Ajaiyeoba et al., 1996). In the US, children belonging to minorities had a significantly higher prevalence of reported visual impairment and blindness than other children. Also, children whose families were below the federal poverty level were nearly twice as likely to be visually impaired as children from families whose income was ≥200.0% of the poverty level. Further, children from high income families were more likely to see an eye-care provider during the preceding 12 months than children from low income families (CDC, 2005). Also, in India the large number of blind children among the rural population was from the lower socioeconomic class (Jain et al., 2005).

Pollution in the house and/or environment is associated with increase prevalence of trachoma (Rashwan and Mohamed, 1992; Ajaiyeoba et al., 1996; Ajaiyeoba & Scott, 2002 and Hesselbarth, 2005). Also, the water-washed diseases (as eye diseases) caused by insufficient water for personal hygiene; children are disproportionally affected (Hesselbarth, 2005). The prevalence of trachoma among those with unclean faces were three times more likely to have clinical trachoma or ocular *C. trachomatis* infection, compared with those with clean faces (OR=3.1, 95% CI: 1.6-6.2 and OR=3.0, 95% CI: 1.4-6.3, respectively). Further, about 75.0% of compounds were within 30 minute of a water source. Also, flies on the face were a risk factor for trachoma but not for *C. trachomatis* infection (Abdou et al., 2007). Also, poor eye hygiene was risk factor for eye infections (Shrestha et al., 2009). On the other hand, the good eye- and environmental hygiene are significant protective factors for eye diseases (OR=0.73, 95% CI: 0.57-0.94 and OR=0.71, 95% CI: 0.56-0.90, respectively). At the same time, never received eye examination is a significant risk factor for eye diseases (OR=2.68, 95% CI: 2.05-3.50). Lack of primary health care and eye services are major risk factors of blindness and visual impairment (The USAID Child Blindness Program, 2007). There is gross lack of eye examination in different parts of rural KSA (Al-Nasser et al., 1989). However, only 66.0% of children ages three to five years old in a group of 102 pediatric practices covering 23 states in the US, received vision screenings. No data on office-based vision screenings for older children is available. Health care providers may be missing opportunities to identify vision problems in children during routine visits. It is estimated that only 5.0-14.0% of children receive eye exams performed by optometrists or ophthalmologists before school entry). In KSA, 3.3% of school children had visual loss due to lack of eye care (Badr, 1982a&b). Further, Badr & Qureshi (1981) and Badr (1982a) cleared that only 9.7% and 8.2%, respectively of their students in KSA had received eye examinations. The prevalence of undetected vision problems among school children in the US was estimated to be 5.0-10.0% (Castanes, 2003). Also, in the US, among children aged 6-17 years; 20.7% had visited an eye-care provider during the preceding year. Asian, non-Hispanic black and Hispanic children (15.0%, 19.1%, and 15.5%, respectively) were significantly less likely to have visited an eye-care provider during the preceding year than non-Hispanic white children (22.8%) (CDC, 2005). So, eye care oriented physician with specific training courses in management of community ophthalmic problems is recommended (Rashwan and Mohamed, 1992). Also, previously undiagnosed eye problems especially refractive errors were found in 22.5% (Adegbehingbe et al., 2005). Lastly, early detection of eye diseases and compliance with treatment is requested (Hunter, 2005). Also, intervention chemotherapeutic is urgently recommended to trachomatous children (Rashwan and Mohamed, 1992), as it shown to be succeeding in reducing infection among children by 4-10 folds in Tunisia (Dawson et al., 1976) and many other places (Melese et al.. 2004; Solomon et al., 2004; Gaynor et al., 2003 and Chidambaram et al., 2006). So, early diagnosis and compliance with therapy is urgently needed.

We cleared that younger age group 6-8 years was insignificant protective factor, and older age group 12-16 years was insignificant risk factor. These results are expected as some of the studied eye diseases are more prevalent among small age group as trachoma and MPC, while other studied eye diseases are more prevalent among big age group as errors of refraction. In addition, rates for vision problems increase as children age. Nearly 8.0% of children ages 0-5 experience eye problems, while 25.0% of adolescents 12-17 are reported to have eye problems). Also, the prevalence of ocular infections was increased with increase in age (Kumar et al., 2004 and Shrestha et al., 2009). There is a preponderance (48.7%) of eye disorders in students aged 13-15 years (Adegbehingbe et al., 2005). Also, Khandekar and Abdu-Helmi (2004) showed that rate of vision impairment was significantly higher among high age group (P=0.0001). We reported that male sex was significant risk factor. This result is expected as many of the studied eye diseases are more prevalent among males (Rashwan and Mohamed, 1992; MOLISA, 1998; Ajaiyeoba & Scott, 2002; Khallaf and Khalifa, 2004; Adegbehingbe et al., 2005; Reddy et al., 2008 and Shrestha et al., 2009). Further, the male sex was significant risk factor for eye diseases (OR=1.6, 95% CI: 1.22-1.51) (Shrestha

et al., 2009). This result could be explained; 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 female sex was risk factor for vision impairment. There are more female students (53.7%) with ocular disorders than males (46.4%), with no statistically significant difference (Adegbehingbe et al., 2005). Girls have less access to medical and surgical services than boys. These services include diagnosis of correctable cataract, treatment of eye infections, and provision of corrective glasses. In a study in Tanzania, parents were less likely to take their young daughters with congenital cataracts to the hospital for surgery than their sons. This gender inequity continues into adulthood; women account for two-thirds of blindness and three-fourths of trachoma-related blindness (The USAID Child Blindness Program, 2007). Further, first and last birth order child were significant protective and risk factors, respectively. These results could be explained; first birth order child is more prone to paternal care while last birth order child is more prone to paternal negligence. Lastly, we noticed that positive consanguinity among parents represented a significant risk factor. This result is consistent with Tabbara et al. (1988) as regard refractory errors and squint. Also, Al-Salem and Rawashdeh (1992); El-Moselhy et al. (2005b) and Tabbara et al. (2005) supported our result and stated that parental consanguinity in those with visual impairment was high. On the other hand, our result is inconsistent with Al-Nasser et al. (1989), they cleared that consanguinity was insignificantly more present among their students with refractory errors (P=0.1). More over, we calculated that their odds ratio risk was 1.17, 95% CI: 0.97-1.41.

Nearly 17 million children with low vision or blurred eye sight lack visual aids, services, or eyeglasses to help them function. These children often are unable to read a chalkboard or textbook. They restrict their movements, fearful of injury or embarrassment. Less than 15.0% of children with disabilities in developing countries have access to education (The USAID Child Blindness Program, 2007). Recent focus on school achievement due to the No Child Left Behind Legislation, Healthy People 2010 recommendations for better child vision screening and expanded computer use among school-age children have re-awakened interest in the importance of childhood vision and early treatment of problems). As refractory errors have found to be associated with headache and difficulties to see clearly what writing on the board in the class room, so it has an adverse effect on the students' scholastic achievement (Al-Nasser et al., 1989). School-based vision screening and eyeglass distribution improve vision and academic potential for children such as these young Guatemalan girls (The USAID Child Blindness Program, 2007).

We observed that small age group and male sex were significant risk factors for trachoma. Our result as regard age is agreed with Rashwan & Mohamed (1992) and Khandekar and Abdu-Helmi (2004). Also, our result regarding sex is in accordance with Rashwan & Mohamed (1992) and Courtright et al. (1989); they showed that the disease is more common among males. This could be explained, the outdoor exposure is more preferable for males and females may have more care for their pictures (Rashwan and Mohamed, 1992). While big age group and female sex were significant risk factors for errors of refraction. Our result as regard age is agreed with Rowe et al. (2004), they showed that vision problems were common and its prevalence increases with age. Also, Khallaf and Khalifa (2004) showed that myopia was more common among girls with big age group (10-19 years), but the difference was insignificant. Moreover, 12.0% of the eleven year old with normal vision show a defect at 16 years (Badr and Qureshi, 1981). Also, El-Moselhy et al. (2005b) agreed that vision impairment was found more among females. On the other hand, Khandekar and Abdu-Helmi (2004) stated that the risk of vision impairment was significantly higher in male students than female. Also, big age group was significant risk factor for allergic conjunctivitis. On the other hand, male sex was insignificant risk factor. Also, Khallaf and Khalifa (2004) reported that allergic conjunctivitis was more common among boys with big age group (10-19 years), but the difference was insignificant. At the same time, Khallaf and Khalifa (2004) cleared that phylecten was more common among girls with small age group (<10 years) with insignificant statistical difference. Also, male sex was insignificant risk factor for squint (Adegbehingbe et al., 2005)

5. Conclusions and Recommendations:

We can conclude that 28.2% of the studied students had eye diseases. The most important eye diseases were trachoma (9.3%), errors of refraction (7.1%) and allergic conjunctivitis (6.3%). All eye diseases were more common in public schools. The most important significant risk factors that for eye diseases were the low level of parental occupation (OR=4.79), previous eye diseases (OR=3.35), no early consultation for eye diseases (OR=3.13), never received eye examination (OR=2.68), sibling(s) with eye diseases (OR=2.19), last birth order child (OR=1.90) and male sex (OR=1.56). Further, age

and/or sex were significant risk factors for specific eye diseases; trachoma, errors of refraction and allergic conjunctivitis. Eye diseases had a significant negative impact on school absenteeism and scholastic achievement of these students; 37.7% had significant school absenteeism 3-4 days/month (P=0.01) and 21.8% had significantly results of the first term exam; <50.0% (P=0.00) compared with their controls. Most of the risk factors of eye diseases can be manipulated. So, many of these diseases and its negative impacts can be prevented. We recommend improving personal and environmental hygienic measures, health education, and regular screening and treatment of students for eye diseases in Egypt. Also, more studies on big number of students in rural and urban areas of Egypt are recommended. Lastly, eye health component of school health services should be focused on and integrated in school health program, and this should be integrated in medical and nursing curriculums.

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The Effect of Tacit Knowledge Characteristics on Tacit Knowledge Transfer: An Empirical Study within Egyptian Industry

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Abstract: The purpose of this research paper is to examine the effect of tacit knowledge characteristics TKC on success factors to tacit knowledge transfer SFTKT from external sources such as suppliers, buyers, universities, and competitors to the recipient of knowledge. This research paper was based on questionnaire survey of Egyptian Industry Sector (75 companies) to investigate the range of attitude and their ability to transfer both organisational and technological knowledge. The questionnaire was carried out by two ways; online, and the great majority via interviews questionnaire. In addition to, the empirical evidence collected from the survey confirms that the urgent need to continuous tacit knowledge transfer process in order to achieve a competitive advantage and sustainability. Additional, results suggest a strong positive effect of tacit knowledge characteristics on success factors to tacit knowledge transfer. As well as**,** empirical study involved the study of the tacit knowledge and classifying it into organisational and technological knowledge depends largely upon functional perspective. This was due to the user diversity**.**

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Key words: tacit knowledge, tacit characteristics, organisational knowledge, technological knowledge, transfer factors, transfer barriers, Egyptian Industries Union.

1. Introduction:

"It is believed that knowledge is qualified to become the main source of wealth in the world. This applies not only to corporations and individuals but also to nations and societies. As individuals and organisations struggle to compete in the global economy, they need more than sound technology; they also must have the support of integrated national and social structures to help them manage their constant demand for new knowledge. Such knowledge – intensive assets include value – creating networks, communities of practice, advisory committees, training and teaching resources". (Parent, Rory, & St-Jacoues, 2007, p.81). Few organisations internally generate all the knowledge required for continuous technological development. And the others must, therefore, often turn to external sources such as suppliers, buyers, universities, and competitors. However, given the tacit and complex nature of most valuable knowledge, its acquisition can be difficult (Kogut & Zander, 1992), Copiedfrom:http://www.accessmylibrary.com/coms2/summary_0286-23920931_ITM. Malik (2004, p.64) clarifies that "there has been a growing realization that successful technology flows in relation to supporting technology transfer and sustaining a firm's competitive advantage depends on the way in which knowledge is generated, articulated, and shared within the organisation" According to Goh (2002,p.25)" the existence of a strong co-operative and collaborative culture is an important prerequisite for knowledge transfer between individuals and groups. Without appropriate mechanisms to encourage co-operation, structured or technological interventions to facilitate knowledge transfer may not work ".

I apply this paper to the Egyptian Industry, particularly companies that benefit from The Industrial Modernisation Centre (IMC), and the Egyptian Industries Union, which are both funded by European Union. This study, I aim to examine the effect of tacit knowledge characteristics on success factors to tacit knowledge transfer, particularly, the availability range or existence of success factors to tacit knowledge transfer in the Egyptian Industry.

Literature Review

Tacit Knowledge Concept

Tacit knowledge concept is simple to depict in digest terminologies but much more evasive when I try to put a concrete and applicable definition. By more clarity, there are many studies focused upon the concept from Polanyi to nowadays. Examples of these studies that are defined tacit knowledge include Polanyi (1966) who adopts philosophical perspective in the definition ; Schön (1983) concentrates upon the importance of transfer of tacit knowledge to 'reflection in action' ; whereas, Vincenti (1990) depicts the contents of tacit knowledge ; while Nonaka (1991) argues tacit knowledge as technical

perspective "know-how" and as elements of organisational culture ; Arora (1996) adds the commercial dimension to the tacit knowledge ; although Smith (2001) repeats some of the perspectives mentioned previously , nevertheless he focuses on tacit knowledge as a personal experience and practice of 'know-how' ; Collis & Winnips (2002) add the technical operational dimension to tacit knowledge ; and finally, Mc Adam, Mason, & Mc Crory,(2007) summarise my viewpoint about tacit knowledge where they combine both technical and organisational knowledge and I have adopted this approach for the objective of the study particularly, they separate and define the two types carefully. Whereas, according to Mc Adam et al., (2007, p.46)" it is necessary to have a workable definition of tacit knowledge. Tacit knowledge is an important element in work and workplace learning and needs to be examined closely in terms of how it is incorporated into organizational practices". Besides," tacit knowledge – knowledge-in-practice developed from direct experience and action; highly pragmatic and situation specific; subconsciously understood and applied; difficult to articulate; usually shared through interactive conversation and shared experience". Depending upon this definition I can conclude that:

Tacit knowledge-knowledge-in-practice developed from direct experience about technological, organisational knowledge; highly pragmatic and situation specific; understood and applied; difficult to articulate; and usually shared through interactive conversation and shared experiences.

Types of Tacit Knowledge

The purpose of this section is to demonstrate which studies concentrate on organisational knowledge or technological knowledge or on both, especially; I adopt the difference between them in order to facilitate the tacit knowledge transfer where the source of these types is variable. As a result of this some of these studies have focused on organisational knowledge like, (Nelson & Winter, 1982; and Inkpen, 1998) where clarify that the acquisition of new organisational knowledge that characterises as tacit and vague. According to Williams (2007, p.869) " there are two key characteristics of organisational knowledge: causal ambiguity and context dependence. Casual ambiguity arises because knowledge is embodied in the repeated activities of the organisation, known as routines. On the other hand, context dependence arises because knowledge integrates components of knowledge – such as people, personal networks, or information – which vary between different settings". Doubtless, this type of knowledge has played a central role at the top of managerial priority in order to gain and sustain competitive advantages for the corporations regard, it serves this level of management to facilitate decision making and solve the managerial problems.

In relation to studies which discuss the technical knowledge 'know-how' like, Bohn (1994, p. 62) that defines technological knowledge as "understanding the effects of the input variables [of a manufacturing process] on the output. Additional, Orlikowski, (1993); and Lapré, Mukherjee, & Van Wassenhove, (2000) concentrate on the effect of technological knowledge on technology performance so as to improve the performance between individuals and groups in manufacturing organisations. This knowledge should be shared and rooted in actions.Moreover, Edmondson, Bohmer, & Pisano,(2001)assure the factor of trust for developing and applying new technology among individuals While, Doz & Hamel (1997) add the environmental dimension whereas technological knowledge is embedded within a specific context. Kachra & White (2008, p.426), gather between the characteristics and trade perspective that "the tacit knowledge is non-proprietary, not protected by patent, does not constitute a firm – specific trade secret, and there is no formal embargo on its transfer. We refer to this type of knowledge – tacit, non-proprietary, and technological – as 'know – how'".In my opinion, this type is useful to apply on technical problems particularly, manufacturing processes.

The rest investigates both technological knowledge and organisational knowledge. For example, Agarwal, Echambadi, Franco, & Sarkar, (2004, p.502) collect the types that, "we consider two specific types of know-how, namely, technological and market pioneering. A firm's technological know-how reflects its ability to generate new scientific discoveries and technological innovations before competitors do". Furthermore, Guzman and Wilson (2005, p.60) clarify that "organisational knowledge is a concept with a wide scope. On the one hand, it refers to socially constructed templates (concepts, methods, routines, techniques and tools) usually used to improve performance. On the other hand, organisational knowledge is also attached to "artifacts "(i.e. equipment, machines) or to "technical processes". In both cases, organisational knowledge is needed in order to adapt either artifacts or technical processes to the specific – social and technical – local conditions of operation, and / or vice versa". I think that the gap in these studies is to mix between organisational knowledge and know-how or technological knowledge; it remedies both of them under one type namely organisational knowledge. By contrast, in this study I separate between them, this is without doubt, represents stepping stone accordingly the source and the user of knowledge that we can get

it from learning by doing, rather than from learning by theory. When regarding with tacit knowledge used to enhance group innovation, Leonard & Sensiper, (1998) clarify that it plays a predominant role in two applications problems discovering and solving, and strategic planning through future events anticipation.

I suggest that the types of tacit knowledge which depend largely upon functional perspective are:

• Organisational knowledge points out socially structured templates (concepts, methods, routines, and tools) and shared company's culture. It often applies to the top level of management and takes managerial priority, in brief management systems.

• Technological knowledge means, know – how, techniques, artifacts, and technical processes as a whole, in brief it relates to equipment, machines, and so on. It often applies to operational management level. Therefore, Figure 1.classify the types of tacit knowledge onto organisational and technological knowledge.

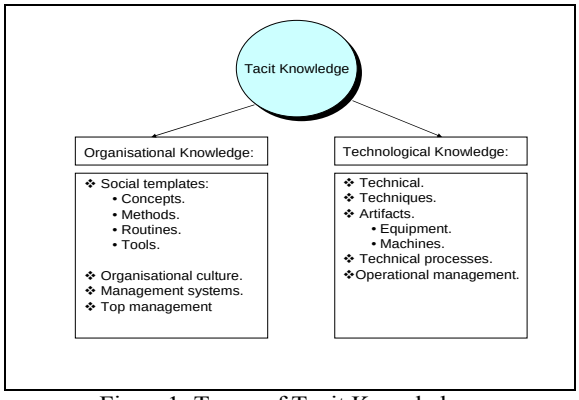

Figure1: Types of Tacit Knowledge

Tacit Knowledge Characteristics

In surveying of tacit knowledge characteristics I focus mainly upon two studies Wong & Radcliffe, (2000) and (Johnson, 2007) because they recite these characteristics with some details and do not mix between tacit characteristics and factors prerequisite for tacit knowledge transfer especially the differences between them resemble a fibre thin. Continuously, tacit knowledge is the term used to illustrate any shape of difficulty in quantifying knowledge, respect the knowledge about social interactions and practices, and more obviously, how an individuals and groups get things done. According to, Linde (2001, p160), "this type of knowledge is considered particularly problematic for knowledge management, because it is difficult to represent as proposition".

Wong & Radcliffe, (2000, p.506) argue in details" through observation, interview, discussion and direct participation processes, the effects imposed on the tasks by knowledge that could hardly be articulated were identified. These effects are regarded as the characteristics of the tacit knowledge, or tacit characteristics, since they help to indicate what tacit knowledge can do and what role it plays in the management and execution of design activities". In order to phrase the appropriate and compatible tacit characteristics from not only simplicity of theoretical frame but also the empirical study, I classify these characteristics into four categories: personal phase which contains personal knowledge and efficiency enhancing; context phase where involves context dependency and trusting in human relationships; available skills where consist of image formation and recognition, judgment facilitating, and physical manoeuvrings and skills; and the final category is available experiences which combine estimation and envisioning and personal experiences that I search for objectivity as shown below and in Figure 2.

Personal phase

• *Personal knowledge.* "The tacit knowledge is a part of an individual's understanding is at once tied to a person's other tacit understandings and may also be shared with others. This shared tacit knowledge is often conceptualised as group or organisational knowledge". (Johnson, 2007 p.126).

• *Efficiency enhancing.* "This resembles the general learning curve model which suggests that efficiency improves in subsequent trials. Some knowledge is involved that contribute to such efficiency enhancement ". (Wong & Radcliffe, 2000, p.506).

Context phase

• *Context dependency*. According to, Augier, Shariz, Vendelo, (2001, p.129) "context is an individual construct and emerges as an individual encounters a situation, including others and artifacts, as it is the individual's interpretation of situation that results in context".

• *Trusting in human relationships.* "These concerns the knowledge used in dealing with people and the realisation of human factors in various circumstances". (Wong & Radcliffe, 2000, p.507). According to Alavi, Kayworth & Leidner (2005,p.197) "a "good" cultural values such as sharing, openness, and trust will lead to positive knowledge management behaviors (e.g., knowledge contribution and sharing), which will lead to innovation and efficiencies".

Available skills

• *Image formation and recognition.* "When working with a design task, the design engineer must

formulate, at the back of his mind, an image of the artifact he is designing. On the other hand, image recognition is also a state of mind that utilizes in articulable knowledge.Have to illustrate the tacit characteristics of the knowledge concerned. As a result, tacit knowledge is an action when we need to formulate organise images". (Wong & Radcliffe, 2000, p.506-507).

• *Judgement facilitating.* "This refers to the knowledge of an opinion about something. In a design project, judgement is required in many yes/no, true/false, positive/negative, go/no go situations. However, how the design engineer makes a certain judgement was beyond articulation. This is the work of tacit knowledge". (Wong & Radcliffe, 2000, p. 506).

• *Physical manoeuvrings.* "These are often referred to as skills. These involve physical body movement and co-ordination, preparing sketches, controlling hand or machine tools and so on are tasks that require tacit knowledge to control body to move at the right time, with the appropriate amount and along the proper orientation in order to achieve the task". (Wong & Radcliffe, 2000, 506).Furthermore, this element forms a part of personal knowledge also, but with some details.

Available experiences

• *Estimation and envisioning capability.* "This requires the envisioning ability of foreseeing the potential problems and realising possible outcomes if certain measures were to be instituted. This involves an understanding of the situation and actively evaluates what the possible outcomes of an event may be". (Wong & Radcliffe, 2000, p.506).

• *Path dependency.* "Development of tacit knowledge inevitably depends on personal experience. This is evident in all of the research examining or purporting to describe tacit knowledge". (Johnson, 2007, p.126). In addition to, Baumard, (2002) discusses the influence of experience on tacit knowledge while it accumulates and is applied as people within the professional firm workers collaboratively with their clients.

Therefore, my perspective of tacit knowledge characteristics is shown in Figure 2.

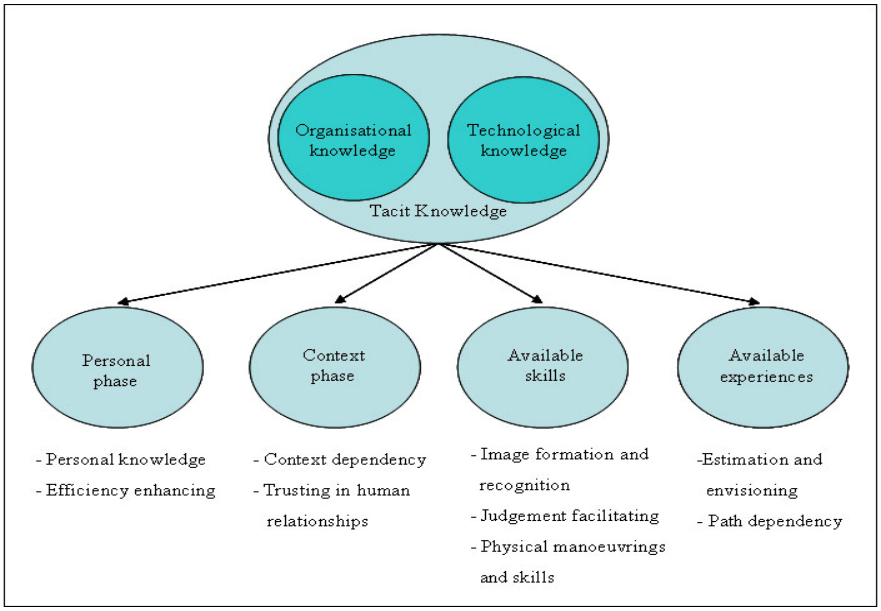

Figure (2): Characteristics of Tacit Knowledge

But without doubt, due to the fact that characteristics of tacit knowledge are stickiness, it may be divided it into two major elements, personal and contextual while the first one involves both experiences and skills.

Tacit Knowledge Transfer

Štrach & Everett (2006, p.62) put in details "the knowledge transfer model where they suggest two dimensions – facilitating factors and knowledge flows" related to Multinational Companies for facilitating knowledge transfer between parent company and subsidiaries . On the one hand, "facilitating factors are contextual conditions that weaken or strengthen knowledge flows; in this model, these are access to knowledge transfer channels, motivation to transfer knowledge, and ability to transfer knowledge". On the other hand, "knowledge flows are spatial and time measurements of knowledge transfer – it is expected that some knowledge enters a subsidiary from its parent

company (inflows), some knowledge is transferred and maintained at the level of the subsidiary (intraflows), and finally some knowledge is exported from the subsidiary to the headquarters (outflows)". While, Nonaka (1994) emphasises that the elements of transfer process are achieved by moving manpower and combining them with tools and management systems. Furthermore, Nonaka & Takeuchi (1995) determine that at least half of all knowledge is tacit and for gaining and transferring it, the work force have working together and trusting each other over a period of time. No doubt, this view point assures the importance of social capital and time dimension in the organisation. Additional, Lei, Slocum, & Pitts, (1997) adopt the same view through emphasising the time have spent and interaction among skilled personnel, particularly, transferring technological knowledge by "day – to – day "activities. Interestingly, Hutzschenreuter & Listner (2007,p.138)epitomise and numerate the three elements of knowledge transfer need to have that include" knowledge transfer context, knowledge transfer fits, and knowledge transfer configuration that are the process which achieves from a sender over a channel to receiver so that it is learned and used". Importantly, Gooderham (2007, p.36) summarises the benefits that "The key element in knowledge transfer is not the underlying (original) knowledge, but rather the extent to which the receiver acquires potentially useful knowledge and utilizes this knowledge in its own operations."

At the end of this argument, I am interested in conditions that facilitate knowledge transfer between firms (intrafirms), and new knowledge, especially knowledge from outside the firms, can be an important stimulus for change and achieve effectiveness and efficiency for the organisation. I think that the studies of Nonaka (1994), Štrach & Everett (2006), and Hutzschenreuter & Listner (2007) represent my view point and may contain the great majority of elements have been required for knowledge transfer.

Factors of Knowledge Transfer

In surveying the knowledge transfer barriers literature, I have focused on thirteen studies dealing with knowledge transfer barriers and /or critical success factors to tacit knowledge transfer. I adopt the historical perspective to present these studies.

On the one hand the barriers include: Nelson & Winter (1982) give three reasons that make transfer of tacit knowledge likely to be more complex; teaching tacit experiences and skills need more time; causal ambiguity; and the knowledge structure needs to have in the organisation. While, Szulanski (1996) introduces new terminologies for transferring knowledge like the source and recipient, he identifies five major barriers to intra – firm transfers of knowledge highlights the two phases, the first, lack of the factors that must avoid in the process particularly, at the recipient and the source like motivation and the degree of relationship between them. The second relevant to the nature of tacit knowledge itself, like difficulty of knowledge. Furthermore, Szulanski (2000) adds some details about knowledge transfer barriers; as stickiness at every stage prerequisite for transferring knowledge, the source reliability, and organisational context. On the contrary, Martin and Salomon (2003, p.363) clarify that Arrow (1969) is the first author mention source and recipient in the process of transfer,"all knowledge transfer events involve both a source, or transferor, and a recipient, or transferee(Arrow, 1969; Szulanski, 1996; Gupta and Govindarajan, 2000)." Although, Ordonez de Pablos (2004, p.111) refers to that "tacit knowledge transfer is complex and difficult for several reasons" like, complex nature, but I think this element is characteristic rather than factor or barricade. Besides, she emphasises the means in which knowledge can be transferred, like learning, teaching, observation, imitation, and organisational learning as a whole. But she does not forget the time dimension to obtaining the knowledge. In addition to, Lesser & Fontaine (2004) identify four barriers to knowledge transfer related to the stages of knowledge management as general which are, awareness , access, application , and perception. Respectively, Sun & Schott (2005, p.81) discuss sources of barriers close individual, organisational, inter – organisational, and team of not only imperative but also climate and relationships. Doubtless, they offer organisational view point in particular, from the side of suitable climate and culture. Whilst, Bou-Llusar & Segarra-Cipre's (2006, p.102) classify the elements of internal knowledge transfer into three categories source unit, recipient unit, and organisational context. But, they introduce five author's models epitomise the relationship between characteristics of knowledge and ease of knowledge transfer process. Dyer & Hatch (2006,p.715) focus on five primary barriers to knowledge transfer involve; "attributes of the knowledge (causal ambiguity / complexity); attributes of the source (lack of motivation and credibility) ; attributes of the recipient (lack of motivation and absorptive capacity) ; attributes of the recipient's existing process (internal process rigidities) ;and attributes of the recipient's network / external environment (network constraints) .Finally, (Schwartz, 2007) groups the main barriers into three categories ; source , recipient ,and organisation as can be seen in table 1.

On the other hand concerning success factors, I can identify three studies, the first one, Goh (2002, p28-29) points out five influencing factors for effective knowledge transfer include;" leadership; problem-solving/seeking behaviours; support structures; absorptive and retentive capacity; and types of knowledge". The second, Reagans & Mc Evily (2003) identify a series of factors critical to successful knowledge transfer focuses upon two types of factors, organisational which remedies the issues of ease and acknowledgement of transfer, and positive relationship between the source and the recipient. As well as, technical or supported like absorptive capacity. The third and finally, (Abou – Zeid, 2005,p.152),suggests model of inter-organizational knowledge transfer intimate culture to facilitate this process" The culturally aware multi-stage model of inter-organizational knowledge transfer identifies eight cultural contexts that play different roles in each stage of inter-organizational knowledge transfer process, namely: the cultural traits of source and recipient firms at four different levels (societal, national, corporate, and operating/occupational)".

I would conclude the theoretical frame by focusing primarily on two dimensions: the first one is the tacit knowledge characteristics which clarifying in figure (2) where divided into both organisational and technological. The second dimension is the success factors or -barriers which must overcome- to knowledge transfer, namely I adopt the study of (Schwartz, 2007, p.253) which concentrates upon three groups of variables combine the process of knowledge transfer and epitomize the main barriers and success factors in table 1.

Table 1 Barriers and/or success factors by category

Source	***Recipient***
1. Ease of transfer	9. Awareness of availability
a. Stickiness at initiation	10. Reliability of the source
b. Stickiness at implementation	11. Motivation
c. Stickiness at ramp-up	12. Available time/Access
2. Ability to transfer	13. Ambiguity of knowledge
3. Willingness to initiate transfer, propensity to share	14. Degree of conjecture
a. Acknowledgement and attribution	15. Absorptive capacity
b. Disseminative capacity	16. Retentive capacity
c. Interpersonal connection	***Organization***
d. Motivation of the source	1 7. Organizational context
4. Awareness of need	18. Organizational design
5. Ambiguity of knowledge	19. Motivation/reward system
6. Available time/Access	20. Available time.
7. Stickiness at integration	21. Nature of relationship between source and recipient.
8.Motivation	

The source: Schwartz,(2007,P.253)

For my empirical study and because I am interested in knowledge transfer at the level of inrafirms (between firms) I choose the Schwartz's (2007) category. Regards to the source of knowledge I pick three elements only; motivation, ability to transfer, and willingness to initiate transfer. I neglect the elements of ease of transfer, ambiguity of knowledge, and stickiness at integration in order to avoid overlapping with tacit knowledge characteristics and in my perspective these elements may similar to some characteristics like personal knowledge and path dependency that I search for objectivity in the study. Moreover, the element of awareness of need already exist in the context of the study. Relevant to the element of available time/access this is depends largely upon the agreement between Europe and Egyptian Government.

The second major factor of knowledge transfer is the recipient; I choose three elements only, reliability of the source, absorptive capacity, and motivation as well as exclude the elements of; ambiguity of knowledge and degree of conjecture as the same reasons mentioned above pertinent characteristics. In addition to, awareness of availability and available time/access are by nature in Egyptian context. And the same view point relevant to absorptive capacity and retentive capacity.

Finally, denote the organisation factor; I define two elements only as expressions on this factor are organisation design (structure) and motivation (reward systems). As the previous discussion I seclude the element of available time like the same reason that mentioned above. Additional, organisational context and nature of relationship between source and recipient that I neglect to avoid

the duplication between success factors to tacit knowledge transfer and characteristics of tacit knowledge.

Doubtless with adoption the barricades this means that I seek overcome these barriers and in accordance with applying the success factors to tacit knowledge transfer. To simplify, I choose the terminology of *success factors* as expression on both. Therefore, my perception of success factors to tacit knowledge transfer as shown in Figure 3.

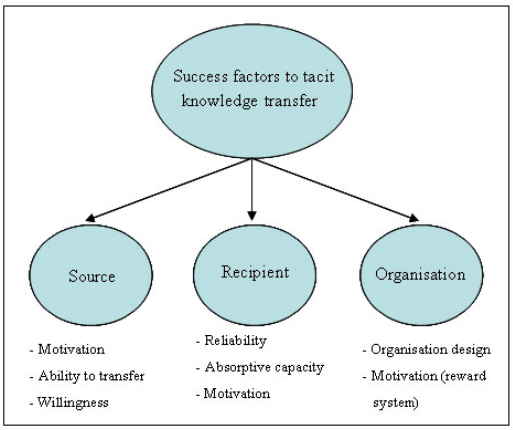

Figure (3) Success factors to tacit knowledge transfer

Then, I can suggest the main research question is as follow:

• Is tacit knowledge characteristics affecting success factors to tacit knowledge transfer? This question may divide into three sub questions like:

1-Is tacit knowledge characteristics (TKC) affecting success factors to tacit knowledge transfer (SFTKT) as source? 2-Is tacit knowledge characteristics (TKC) affecting success factors to tacit knowledge transfer (SFTKT) as recipient 3- Is tacit knowledge characteristics (TKC) affecting success factors to tacit knowledge transfer (SFTKT) as organisation? I think I can find a probably answer for these questions in the empirical study.

Empirical Study

The population

Egypt's economic development critically hinges upon the competitiveness and growth of the industrial sector. Consensus is that industry the engine of growth for Egypt to prosper in the future. The Egyptian Industries Union roughly includes 816 great companies divided into ten sectors which classify under private sector these are: Engineering; Manufacture of Building Materials and Construction Sector; Manufacture of Chemicals; Manufacture of food and Beverage; Manufacture of Leather and Shoes; Manufacture of paper, Printing, and Packaging; Manufacture of wood and Furniture; Pharmaceuticals; and Service related to Industry. Population size consists of seventy five companies that which belong to the private sector, represents the previous sectors and benefits from the Industrial Modernization Centre (IMC) at the first stage of modernising. On the occasion of, these companies are considered not only the greatest companies in its field but also a pioneer in the field of export to foreign countries. In addition the Egyptian Industries Union (EIU) is also supervising these companies. The EIU has funded many centres in Egypt including Information Centre (IC) and (IMC).

The Industrial Modernization Centre (IMC) is intended to perform as the prime agent for substantiating the government's vision of a vibrant and globally competitive industrial sector. The aim is to create an enabling environment in which the private sector can lead growth and make Egyptian industries leapfrog into global competitiveness. IMC was established by a presidential decree number 477/2000 as an independent body to implement and coordinate the modernization of the Egyptian Industry. Jointly funded by the European Union (250 million), the Government of Egypt is (103 million) and the Egyptian Private Sector (73 million) with a total budget of 426 million. Today IMC is part of the everlasting process of continuously bringing the Egyptian industry to international competitiveness. At the first stage of modernizing these companies the (IMC) have been adopted the developing and improving managerial and organizational abilities of human resources to seventy five greatest companies represent all sectors, this process occurs both in Egypt and Europe. Additional, having improved the technological abilities for technical supervisors in these companies, this is through training them and sharing knowledge at thirty industrial training centers in Egypt. In brief, I can determine that the population under study is the human resources at 75 companies.

The sample

The population size contains 75 companies; these companies roughly have a total labour size of 37,500. This is a sampling frame and because of this frame is a huge to study, I calculated the sample size according to sample for proportions formula, Cochran, (1963:75) with 95% confidence level and 0.05 variation, then sample size is, 385. I gathered sample data from line, top and staff managers, furthermore, technical workers at the plants where these labour have a contact with foreign expertise from European Union. I focused our investigation on people who have benefited from training courses as much as I can. These courses as previously mentioned, I consider it as a tacit knowledge transfer

(organisational and technical) in order to recognise the range of advancements in these companies particularly, with respect to their tacit knowledge. Moreover, it will help to better understand the attitude towards the success factors to tacit knowledge transfer

Questionnaire Development

I develop the questionnaire of tacit knowledge characteristics based on these studies (Nonaka & Takeuchi, 1995; Wong & Radcliffe, 2000; Augier, Shariz, Vendelo, 2001; Baumard, 2002;Alavi, Kayworth & Leidner 2005;and Johnson,2007). Concerning the questionnaire of success factors to tacit knowledge transfer, I quote these factors or barriers from four studies, (Szulanski, 2000; Goh, 2002; and Reagans & Mc Evily, 2003.Besides, I adopt the study of (Schwartz, 2007) which concentrates upon three groups affect the process of knowledge transfer and he epitomizes the main barriers and success factors as shown in table 1. Furthermore, multi- item scale based on established measures were used for almost all the constructs within the questionnaire and were measured on the same five-point Likert-type scale ranging from 1 ("strongly disagree") to 5 ("strongly agree"). As can be seen in Appendix 1 using Cronbach's alpha (α) as a measure of reliability.

Research Methodology

The Objectives

Tacit knowledge transfer takes a central role in the study. Therefore, adequately having measure the transfer that occur within an organisation, as well as from outside the firm is of vital importance to this work. Thus, I need to measure the degree of an attitude the research units toward success factors to tacit knowledge transfer among firms. Hence, the objectives of the empirical analysis were (1) to describe characteristics of tacit knowledge, (2) to measure the attitude towards the success factors to tacit knowledge transfer, (3) to test the proposed hypotheses and thus the effect of tacit knowledge characteristics on success factors to knowledge transfer. Descriptive statistics are used to meet the first and the second objectives, and Step-wise Regression analysis is performed to meet the third objective.

Data Collection

Data for this study were collected through a questionnaire survey directed at line, top, and staff managers besides technical workers in every company were defined in sample size. The data for this study were collected during the second quarter of 2009 using two ways, online and interviews questionnaire particularly the great majority of companies under study locate in new industrial cities like Tenth's of Ramaddan City, Sixth's of October City, Aloboar City, New Aamireia City, and Al Sadaat City .This concentration of these companies facilitate and accelerate gathering data process. The questionnaire were written and answered for the purpose of this paper, and I translated it into Arabic language.

Sample Data

The target group consisted of 385 employees from line, top and staff managers, in addition to technical workers who have benefited from training courses. A total of 327 surveys were returned, I secluded 9 surveys uncompleted, which means that the accepted returns equal 316 representing a response rate 82 %.

Dependent Variable

For practical reasons and statistical results accuracy, including the ease of measurement, I put success factors to tacit knowledge transfer on 8 statements divided onto 3 major elements, source, recipient, and organisation.

Independent Variables

The independent variable of the present study are predominantly based on pre-existing constructs, I form characteristics of tacit knowledge in 9 statements divided onto 4 major variables, personal phase, context phase, available skills, and available experiences.

Hypotheses of the Study

From the previous dependent and independent variables, the main research questions, and the proposed affect is captured in the final set of hypotheses as follow:

Hypothesis 1: There is no effect of tacit knowledge characteristics on success factors to tacit knowledge transfer (source).

Hypothesis 2: There is no effect of tacit knowledge characteristics on success factors to tacit knowledge transfer (recipient).

Hypothesis 3: There is no effect of tacit knowledge characteristics on success factors to tacit knowledge transfer (organisation).

Validity and reliability

In the design of the questionnaire, the following steps were taken to ensure measurement validity and reliability. First, the initial constructs of the questionnaire were based on a thorough literature review. Second, the questionnaire was pre-tested by

experienced academics in the field of Human Resource Management. Third, a personalised cover letter accompanying each questionnaire explained the purpose of the study, provided assurances regarding the confidentiality of the collected data, and results of validity and reliability as can be seen in Appendix 2. Finally, I measure reliability by using Interconsistencey and Cronbach's alpha (α) for both tacit knowledge characteristics and success factors to tacit knowledge transfer. But I found that I have a problem of multicolleaniarity between tacit knowledge characteristics as independent variables at the pilot study stage as shown in Table 2. I have not only assured on it through Correlation Matrix for the final data but also neutralised multicolleaniarity by using Step-wise Regression model for analysing data. Importantly, having confirmed that estimated models are not suffering from this problem through proven that all signals of estimated models parameters are positive accordingly the theoretical frame particularly between the two variables (independent and dependent).

Table 2 Correlation matrix between variables of tacit knowledge characteristics

Variables of the study	Personal phase	Context phase	Available skills	Available experiences
Personal phase	1	0.671**	0.967**	0.480**
Context phase		1	0.716**	0.626**
Available skills			1	0.494**
Available experiences				1

(**) refer to Person's correlation (two-tailed) (r) is significant at (0.01).

According to correlation matrix as can be seen in Table 2 I get that there are interrelationship between the four explanatorily variables and all of them are positive, highly correlated, and highly significant.

Results

Descriptive statistics for all of the variables analysed in this study are provided in Table 3 that denote high degree of responses and variability between values is small**.** Hypothesis of the study were tested using Step-wise Regression Model. Data were carefully examined with respect to linearity, equality of variance and normality by plotting standard residuals against predicted values. No serious deviations were detected. Data were also carefully examined for multicollinearity among the independent variables notwithstanding at the pilot study stage but I dealt with its effects by using appropriate statistical model to analyse the data for examined the hypotheses is as follow:

Table 3. Descriptive statistics

Variables of the study	Descriptive statistics	
	Mean	Coefficient of variation
Independent variables		
Personal phase	4.02	21.69
Context phase	4.35	14.29
Available skills	4.11	19.23
Available experiences	4.04	17.04
Dependent variables		
SFTKT(source)	4.08	15.19
SFTKT(recipient)	4.25	10.59
SFTKT(organisation)	4.19	14.57

The first hypothesis

I can put two stages to judge this hypothesis as the following:

(1) Studying the relationship between SFTKT (source) and TKC. The results shown in Table 4 where indicative to, SFTKT (source) were represented by motivation, ability to transfer, and willingness is highly positive correlated with the different variables of TKC namely, personal phase, context phase, available skills, and available experiences. Besides, this relationship is highly significant at level 0.01.

(2) Estimation the degree effect of TKC on SFTKT by using Step-wise Regression technique because I found interrelationship between the four variables of TKC. With using Step-wise regression the multicollinearity problem may be solved and getting the best combination from TKC that affects SFTKT (source) as shown in model 1.

Table 4. Correlation coefficient between success factors to tacit knowledge transfer (source) and tacit knowledge characteristics

Dependent variables / Independent variables	SFTKT(source) Y1T
Personal phase (X1T)	0.923**
Context phase (X2T)	0.774**
Available skills (X3T)	0.952**
Available experiences (X4T)	0.633**

(**) refer to Person's correlation (two-tailed) (r) is significant at (0.01).

This model denote that SFTKT (source) were represented by motivation, ability to transfer, and willingness is affected by available skills, available experiences, and context phase respectively (R^2 = 0.944) are highly significant and strong

supported at the 99% level and *F* – value at 1785.93.Also, the standard error was at the lower level. This clarify that all of the independent variables which mentioned in the present model interpret 94.4 % of variations in SFTKT (source).

Model 1 for the first hypothesis

Y1T = 0.461 + 0.627 X3T + 0.165 X4T + 0.086 X2T
(7.656)** (41.884)** (10.749)** (4.050)**
F – Value = 1785.93** , with *d. f.* (3 ‘ 316)
R^2 = 94.4 % , S.E = 0.147

(**) refer to significant level at (0.01) for the test statistic (F & T) values.

From the above discussion I can reject the first hypothesis and then:

There is effect of tacit knowledge characteristics on success factors to tacit knowledge transfer (source).

The second hypothesis

According to statistical analysis for this hypothesis I can define two stages also:

The first related to examine the relationship between SFTKT (recipient) and TKC whereas the results shown in Table 5 have been proved that the dependent variables SFTKT from recipient side were represented through reliability of the source, absorptive capacity, and motivation is moderate positive correlated with the different variables of TKC namely, personal phase, context phase, available skills, and available experiences. Additional, this relationship is highly significant at level 0.01.

Table 5. Correlation coefficient between success factors to tacit knowledge transfer (recipient) and tacit knowledge characteristics

Dependent variables / Independent variables	SFTKT(recipient) Y2T
Personal phase (X1T)	0.403**
Context phase (X2T)	0.446**
Available skills (X3T)	0.418**
Available experiences (X4T)	0.404**

(**) refer to Person's correlation (tow-tailed) (r) is significant at (0.01).

The second stage highlight estimation the degree effect of TKC on SFTKT by using Step-wise Regression Model, I am getting the best combination from TKC that affect SFTKT (recipient) as shown in model 2.

Model 2. for the second hypothesis

Y2T = 2.699 + 0.141X2T + 0.125X4T + 0.105X3T
(16.728)** (2.480)* (3.034)** (2.605)**
F- value = 33.431** , with *d.f.* (3 ‘ 316)
R^2 = 24.1 % , S.E. = 0.393

(**) refer to significant level at (0.01) for the test statistic (F & T) values.
(*) refer to significant level at (0.05) for the test statistic (T test) only.

This model demonstrate that SFTKT (recipient) were represented via reliability of the source, absorptive capacity, and motivation is affected by context phase, available experiences, and available skills successively (R^2= 0.241) are significant at the 95 % level relevant to the first variable but I found that are highly significant and strong supported at 99 % level with the other two variables and *F* value equal 33.431. Similarly, I found that the S.E. at lower level. Despite, the weak effects of TKC except if the relationship is significant. From the previous analysis I can reject the second hypothesis and then:

There is effect of tacit knowledge characteristics on success factors to tacit knowledge transfer (recipient).

The third hypothesis

The results related to this hypothesis indicate that:

There is relationship between SFTKT (organization) and TKC, where findings refer to highly positive correlated between dependent variables SFTKT (organization) were represented by organization design(structure) and motivation (reward system) and independent variables TKC where include personal phase, context phase, available skills, and available experiences as can be seen in table 6.

Table 6 Correlation coefficient between success factors to tacit knowledge transfer (organization) and tacit knowledge characteristics

Dependent variables / Independent variables	SFTKT(organization) Y3T
Personal phase (X1T)	0.865**
Context phase (X2T)	0.642**
Available skills (X3T)	0.865**
Available experiences (X4T)	0.502**

(**) refer to Person's correlation (two-tailed) (r) is significant at (0.01).

Accordingly, Step-wise Regression Model I get the best combination from TKC that affect SFTKT (organisation) as shown in model 3. This model clarifies that SFTKT (organisation) were represented through organization design (structure) and motivation (reward system) is affected by personal phase, available skills, and available experiences consecutively (R^2 = 0.769) are highly significant and strong supported at the 99% level and *F* – value at 349.837. As a result, the standard error was at the lower level.

At the end of this analysis I can reject the third hypothesis and then:

There is effect of tacit knowledge characteristics on success factors to tacit knowledge transfer (organisation).

Model 3 for the third hypothesis

Y3T = 1.361 + 0.309X1T + 0.302X3T + 0.087X4T
(12.247)** (4.163)** (3.657)** (3.140)**
F – value = 349.837** , with *d.f.* (3 ‘ 316)
$R^{2=}$ 76.9 %, S.E. = 0.295

(**) refer to significant level at (0.01) for the test statistic (F & T) values.

At the end of this argument I can conclude that these hypotheses were supported by the empirical analysis.

Discussion

The main contribution of this study is to apply and assess the theoretical frame on the Egyptian Industry specifically, private sector. I seek the effect of tacit knowledge characteristics on success factors to tacit knowledge transfer. In fact, the empirical evidence supported the attitude toward tacit knowledge transfer from many sources were mentioned previously, that have a greater impact on performance effectiveness and gain sustain competitive advantages to the companies.

Interestingly, this study contributes to the tacit knowledge literature through suggesting the two types of tacit knowledge which differentiate between organisational knowledge related to top management decisions and technological knowledge that pertains line management from perspective of usage to both. The sample data contains the three different levels; line, top, and staff managers besides technical workers because of the dependence of tacit knowledge transfer in their work. In addition, responses to SFTKT (source) were represented by motivation, ability to transfer, and willingness is affected by available skills which contain - image formation and recognition, judgement facilitating, and physical manoeuvrings and skills - this means that this is the first independent variables impacts on SFTKT (source). Moreover, the available experiences include not only estimation and envisioning but also path dependency that comes at the second degree of influence which encourages the transfer process. But, Song, Almeida, and Wu (2003) treat and test path dependency as the factor of knowledge transfer with patent, therefore, the relationship is negative and significant, this doubtless, stresses my view point that this is no more than explanatorily variable. In relation to, context phase as the third degree of influence which consists of context dependency and trusting in human relationships, this no suspect assure research standpoint and agree with theoretical frame. Pertinent, context dependency, Persson (2006) and Williams (2007) remedy and test this element as independent variable to facilitate the process of tacit knowledge transfer; their results are found strongly supported and highly significant at the 99% level. Furthermore, relative trust in human relationships, all of Li (2005); Dhanaraj, Lyles, Steensmaz, and Tihanyi (2004); Szulanski, Cappetta, and Jensen (2004); and Foos, Schum, and Rothenberg (2006) treat and examine trust as the element of knowledge transfer, in turn, their findings are found highly significant and strongly supported.

Originally, the results reported here indicate that SFTKT (recipient) were represented via reliability of the source, absorptive capacity, and motivation is affected by three independent variables. The first one is context phase that involves context dependency and trusting in human relationships, this clarify that SFTKT (recipient) entails context of tacit knowledge transfer as prerequisite characteristic in spite of the level of significant is not high. Worthwhile, on the one hand, it may reflect the poor of reliability of the source alike from suppliers, buyers, universities, and competitors. Martin and salomon (2003) introduce mathematical model cover source and recipient transfer capacity (absorptive capacity). Respectively, it also probable epitomizes the weak absorptive capacity and motivation at the recipient namely, Egyptian Companies, as a result of this, it should be enhancing these factors. Moreover, Szulanski, Cappetta, and Jensen (2004); and Ambos, Ambos, and Schlegelmilch (2006) remedy and examine absorptive capacity as the factor of tacit knowledge transfer and then as dependent variables where the findings are strongly supported. Likewise, Riusala and Smale(2007) treat the absorptive capacity as a characteristic of tacit knowledge (dependent variable) regression coefficient has negative

relationship with difficulty of knowledge transfer (independent variable) this is no doubt, assure the work view point that absorptive capacity must be treated as a dependent variables particularly, when we talk about knowledge transfer. But, Tsai (2001) have remedied and tested absorptive capacity as explanatorily variable with innovation and performance where the results are strongly supported. On the other hand, regarding with, context phase should be also quite. The second is available experiences that contain estimation and envisioning and path dependency, not surprisingly, this variables occupies the same degree of influence like SFTKT (source) I interpret this result as probable weak recipient specifically its sub ingredients. The third is available skills which comprise image formation and recognition, judgement facilitating, and physical manoeuvrings and skills this embody the physical side of knowledge which accelerate the transfer process.

Importantly, I found that SFTKT (organisation) were represented through organization design (structure) and motivation (reward system) is affected by personal phase, available skills, and available experiences. Inconsistency, with this result Cheng (2005) finds that motivation as endogenous variable is not significant vice versa skills is highly significant, whereas, reward as exogenous variable is highly significant. Whilst, Szulanski, Cappetta, and Jensen (2004) focus upon some factors like source's motivation, organisational context, trustworthiness, recipient absorptive capacity, and recipient's motivation; and they conclude that the first four factors are significant ranging between moderate and highly significant. Relevant, personal phase I notice that despite the argument focuses here upon the organisational factors except if both personal knowledge and efficiency enhancing, come at the first degree of impact, in my opinion this reflects the necessary need to relate these factors with organisation structure I mean by promotion and create position for knowledge management on formal organisation structure. By the way, The Egyptian Cabinet- Information Decision Support Centre adopts the latest recommendation to apply on companies in Egypt. Furthermore, available skills and available experience cover the couple side of knowledge material (skills) and immaterial one (experience). Obviously, the triangle influence upon these dependent are highly significant, in turn, this finding elicit the important of organisation factors to tacit knowledge transfer. Finally, I can conclude this argument by answering for the main research question that tacit knowledge characteristics (TKC) are affecting success factors to tacit knowledge transfer (SFTKT) as source, recipient, and organisation.

Conclusion

The aim of this study was to examine the relationship between tacit knowledge characteristics and success factors to tacit knowledge transfer specifically, the effect of TKC on SFTKT. I found that The Industrial Modernization Centre (IMC) has an effective role in the field of tacit knowledge transfer from many sources; suppliers, buyers, universities, and competitors. Obviously, in relevant to the level of adoption initiative and the level of implementation, were at each site in all companies. These companies get (transfer) their tacit knowledge from many sources. This depends on the classification which I have covered about types of tacit knowledge from a functional perspective:

- Organisational knowledge transfer to these companies from Universities (staves in all industrial and managerial areas) where work with Private Expert Houses that are funded by (EIU-CMI) European Union.
- Technological knowledge transfer to these companies is from two sources, on the one hand, from equipment suppliers (UK, Germany, France, and Italy) and expertise (employees) for long time from European Union. On the other hand the tacit knowledge comes from expertise and technical professional from competitive companies but by informal way.

The results depend largely upon the responses about the success factors to tacit knowledge transfer that were found significant with tacit knowledge characteristics. This means that both managers and technical workers have a recognition to the necessary of tacit knowledge transfer despite the tacit knowledge characteristics may a barrier. Results largely support the proposed model. These results, I suggested, have important implications for practitioners and further scholarly research alike. Also, as to the practical implications, the study can be used as a source of recommendations for companies to enable them to overcome some of the potential difficulties and problems in knowledge transfer.

For the theoretical frame and how managers can use the tacit knowledge both organisational and technological. I separate between these knowledge specially many of studies blend between them as (Nonaka,1991; Gupta & Govindarajan, 2000; and Guzman & Wilson, 2005) where they mentioned that every tacit knowledge for example technological(technical or know-how) is organisational knowledge but I propose that clarify the differences between them accordingly the level to create the knowledge where organisational

knowledge quoted from top level in the organisational structure and probable useful for planning and managerial decision making. While technological knowledge that I obtain from middle or first line management and may use this knowledge in both higher and lower levels but mainly in the lower level. I can complete that I am interested in tacit knowledge transfer process among individuals at the level of intrafirms through training, moving people (expertises from European Union), and sharing knowledge despite the tacit nature of knowledge. And my aim from the study may be achieved completely.

Actually, this study contributes in several aspects for tacit knowledge characteristics and transfer, it also has important limitations. Specifically, there are two limitations affecting the scope of these findings. The first limitation is that our survey respondents were representatives only from the private sector (vertical comparison), but I needed to compare between public sector and private ones to complete the judgment process transfer particularly the effectiveness measures (horizontal comparison). The second limitation was the limited number of factors related to tacit knowledge transfer which I have examined. Future research would benefit from public sector as well as private sector and examine more factors to tacit knowledge transfer.

Future Directions

It has recently been suggested that it is preferred to study the success factors to knowledge transfer in detail where one can include the main three elements to transfer process; the source; the recipient; and the organisation (Schwartz, 2007). I finish by suggesting directions and topics for future research. Therefore I propose three titles of studies that would be useful in this field; the first is: Evaluating the Relationship between Tacit Knowledge Characteristics and the Source of Knowledge; the second is: Evaluating the Relationship between Tacit Knowledge Characteristics and The Recipient of Knowledge; and the third is: Evaluating the Relationship between Tacit Knowledge Characteristics and The Organisational Factors. As well as, go deepen to organisational factors namely trust in knowledge sharing and then the title may be: Does Trust Enhance Knowledge Sharing among Individuals within the Organization? Each of the studies will be expected to follow a similar methodology as outlined in my approach in this paper.

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Appendix 1
The questionnaire

Dependent variables
Success factors to tacit knowledge transfer •**Source** - Motivation. - Ability to transfer. - Willingness. •**Recipient** - Reliability of the source. - Absorptive capacity. - Motivation. •**Organisation** - Organisation design (structure). - Motivation (reward system).
Independent variables
Tacit knowledge characteristics •**Personal phase** - Personal knowledge. - Efficiency enhancing. •**Context phase** - Context dependency. - Trusting in human relationships. •**Available skills** - I mage formation and recognition. - Judgement facilitating. - Physical manoeuvrings and skills. •**Available experiences** - Estimation and envisioning. - Path dependency. Not: All variables were measured on five-point Likert- type scale ranging from 1 (“Strongly disagree”) to 5 (“Strongly agree”).

Appendix 2

Table 1 Validity and reliability of tacit knowledge characteristics

Statements	Personal phase	Context phase	Available skills	Available experiences
- Personal knowledge x_1	0.919**			
- Context dependency x_2		0.913**		
- Efficiency enhancing x_3	0.894**			
- Image formation and recognition x_4			0.864**	
- Trusting in human relations x_5		0.911**		
- Judgment facilitating x_6				
- Estimation and envisioning x_7			0.892**	
- Path dependency x_8				0.849**
- Physical maneuverings and skills x_9				0.829**
			0.675**	
Chronbach's Alpha coefficient (α)	0.785	0.797	0.742	0.578

(**) the value is significant at (.01)

Table 2 Validity and reliability of success factors to tacit knowledge transfer

Statements	Personal phase	Context phase	Available skills
- Motivation Y_1	0.827**		
- Ability to transfer Y_2	0.863**		
- Willingness Y_3	0.741**		
- Reliability of the source Y_4		0.647**	
- Absorptive capacity Y_5		0.722**	
- Motivation Y_6		0.455**	
- Organisation design (structure) Y_7			0.897**
- Motivation (reward system) Y_8			0.938**
Chronbach's Alpha coefficient (α)	0.731	0.152	0.802

(**) the value is significant at (.01)

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Evaluation of an experimental zinc phosphate cement powder

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Abstract: The aim of this study was to evaluate the properties of an experimentally prepared zinc phosphate cement powder. The working time, setting time, film thickness, compressive strength and solubility were tested for the experimental cement powder and compared with one of the commercially available zinc phosphate cement. Testing was done according to the ANSI/ADA specification No. (8) for zinc phosphate cement and No. (96) for dental water-based cements. Results revealed that the experimental cement produced working time, setting time, film thickness and solubility comparable with that specified by the ADA specification No. (8) and (96), and with that of the commercial cement, however the compressive strength (42.09 MPa) was significantly lower than that specified by the ADA No.(96) (70 MPa) but was not significantly different than that of the commercial cement (49.6 MPa).
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Key words: zinc phosphate cement, ANSI/ADA specification No.(8) and No.(96), working time, setting time, film thickness, compressive strength, solubility, disintegration.

1. Introduction

A number of materials are available for cementation, luting and bonding purposes in dentistry. Zinc phosphate cement has been used for the longest period compared with other cement types.

Dental zinc phosphate cement is supplied in the form of powder and liquid. The typical formulation of zinc phosphate cement powder is zinc oxide (90.2%wt), magnesium oxide (8.2%wt), silicon dioxide (1.4%wt), bismuth trioxide (0.1%wt) and other minimum ingredients e.g. BaO_2, Ba_2SO_4 and Ca O (0.1%wt). However, the liquid is free phosphoric acid (38.2%wt), phosphoric acid combined with aluminium and zinc (16.2%wt), aluminium (2.5%wt), zinc (7.1%%wt) and water (36.0%wt). Craig et al.,(2004);(2006) .The set zinc phosphate cement is essentially a hydrated amorphous network of zinc phosphate that surrounds incompletely dissolved particles of zinc oxide. Although no crystalline phosphate is involved in the setting process of the cement, there can be subsequent growth of crystalline hopeite, ($Zn_3(PO_4)_2$. $4H_2O$) in the presence of excess moisture during setting, Craig et al.,(2006).

The initial hardening of zinc phosphate cements normally occur within 4-7 minutes, although the strength continues to increase for some time after that. The ADA specification No.(96) American Standards Institute (1977;1994) carries no requirement for the working time, so the results of the working time test of the commercial cement were taken as reference to the experimental cement. However, the ADA specification No.(96) stated that the net setting time is 2.5 minute minimum and 8 minutes maximum. Therefore, the total setting time (working time + net setting time) would be 7.5 minutes minimum and 13 minutes maximum, Walls and McCabe(1998).

The desired consistency of the zinc phosphate cement mixture depends on the particular purpose of the material and the working convenience needed, as expressed by the setting time, where the inlay seating require maximum film thickness of 25µm i.e, light consistency, American Standards Institute (1994). However, the cement base should have a heavier consistency to be used as a thermal and chemical insulating barrier over thin dentin Craig et al.,(2004).

The ADA specification No.(96) stated that the compressive strength of zinc phosphate cement should be 70Mpa minimum and that the disintegration percentage should be 0.1 % maximum, American Standards Institute(1994)This study was designed to compare the properties of an experimentally prepared zinc phosphate cement powder with a commercially available one, and to evaluate their properties according to the ANSI/ADA specification No. (8) for zinc phosphate cement and No. (96) for dental water-based cements.

2. Materials and methods

2.1. Materials:

2.1.1. Cement powder:

One of the commercially available zinc phosphate cement powder (Alpha-dent, Dental technologies manufacturer, USA) was selected to

compare its properties with that of the experimental cement powder.

An experimental zinc phosphate cement powder was prepared according to Safwat et al (2007) The chemical composition of the experimental cement powder is listed in table (1).

Table (1): The chemical composition of the experimental cement powder.

Zinc oxide	Magnesium oxide	Aluminum oxide	Silica	Bile bovine	Borax
91.66 %	2.2%	3.31%	0.126%	1.43 %	0.9%

2.2.Methods:

2.2.1.Sample preparation:

Preparation of the commercial cement samples was done according to the manufacturer instructions, using liquid to powder ratio (L/P) of 3:1. On the other hand the experimental cement samples were prepared according to Safwat et al (2007) using L/P ratio of 3:1.

2.2.2. Evaluation of the physical and mechanical properties:

2.2.2.1. Evaluation of working time:

A teflon ring mold approximately 4.8mm high and 9.5mm internal diameter was used to prepare the cement specimens. An indentor of 28gm weight, having a needle with flat end of 2mm diameter was used. The tip of the indentor was cylindrical for approximately 5mm, plane and perpendicular to the long axis of the needle. A holding ring was also used to insure vertical and perpendicular loading of the indentor. The working time was measured as the time elapsed from beginning of mixing till the needle no longer penetrates the surface. The test was repeated five times; the mean and standard deviation (S.D) of both the experimental and the commercial zinc phosphate cement were calculated.

2.2.2.2.Evaluation of setting time:

The same teflon ring mold used for the working time evaluation was used to prepare specimens for the setting time testing. An indentor of 400gm weight, having a needle with flat end of 1mm diameter was used. The needle tip was also cylindrical for approximately 5mm, plane and perpendicular to the long axis of the needle and a holding ring was used to insure vertical and perpendicular loading of the indentor. Three and half minutes after starting of mixing, the indentor was carefully lowered vertically through the holding ring onto the surface of the cement in the ring mold, left for five seconds under its own weight then a trial run was carried out. This procedure was repeated at 30 seconds intervals until the needle failed to make a complete circular indentation on the surface of the specimen. The setting time was recorded as the time elapsed from the start of mixing till the needle failed to make a complete circular indentation on the surface of the specimen. The setting time was recorded to the nearest minute and the test was repeated five times. The mean and standard deviation for both the experimental and the commercial zinc phosphate cement were calculated.

2.2.2.3.Evaluation of film thickness

The thicknesses of two flat square glass plates of 5mm uniform thickness and 45mm length, having contact surface area of 200mm^2 were measured while stacked in contact using a digital micrometer accurate to the nearest 1.25mm. The cement mixtures were then mixed and placed between the two plates. Ten seconds before the previously determined working time, a loading device with a force of 15Kg was applied vertically and centrally on the upper glass slab for ten minutes. The thicknesses of the two plates were measured with the mixed cement in-between. The film thickness was calculated as the difference between the two measurements. The test was repeated five times; the mean and standard deviation for both the experimental and the commercial zinc phosphate cement were calculated.

2.2.2.4.Evaluation of solubility and disintegration:

Two empty graduated glass beakers were thoroughly dried and weighed. Four cylindrical specimen, 20mm diameter and 1.5mm thick, were prepared using a metal mold, three minutes after mixing, specimens were stored for 1 hour in a relative humidity of 100% at 37°C. The specimens were then submerged in the two beakers containing 50ml-distilled water each for 23 hours at 37°C then removed from the beakers, leaving behind their remnants in the distilled water. Distilled water was then completely evaporated at temperature just below its boiling point i.e., approximately 90°C, leaving only the remnants of the cement in the beakers. The two beakers were then reweighed and the amount of disintegration was calculated as the difference between the two weight measurements. The weight gained by the beakers divided the original weight of the specimens' multiplied by 100 gives the percentage of disintegration reported to the nearest 0.1%.

2.2.2.5.Evaluation of compressive strength:

A split teflon circular mold 6mm in height and 4mm in diameter with a holding ring to hold the mold plates together was used. The largest convenience portion of the cement is applied to one side of the mould 60 minutes after the end of mixing and filled in excess to consolidate the cement and avoid trapping air; another glass slab was applied over the mold to obtain a smooth surface. Five specimens were prepared then removed from the mould after one hour and immediately immersed in distilled water at 37°C for 23 hours. Twenty-four hours after the end of mixing, the prepared samples were individually and vertically mounted on a computer controlled universal testing machine (model LRX-plus; Lloyd instruments Ltd., Fareham, UK) with a load cell of 5KN and a crosshead speed of 0.75mm/min. The load at failure was recorded and the compressive strength values in MPa were calculated for five replicas; the mean and standard deviation for both the experimental and the commercial zinc phosphate cement were calculated.

2.2.3. Statistical analysis:

The mean and standard deviation were recorded and statistically analyzed using ANOVA test to compare the ranks of the levels of the experimental and commercial groups. SPSS for windows software, release 15.0 (SPSS, Chicago, IL) was used.

3. Results

3.1. Results of working time, setting time and film thickness:

Results of working time, setting time and film thickness are shown in table (2). Regarding the results of working time, no statistical significant difference could be detected between the experimental cement and the commercial cement (5 and 4 minutes respectively) (P=0.4). Results also indicated that both the experimental and the commercial cement have the same setting time (7.5 min), which meets that specified by the ADA specification (96). American Standards Institute (1994). As regards the film thickness, both the experimental and the commercial cements have the same film thickness (25µm) which also meets that specified by the ADA specification (96), American Standards Institute (1994).

3.2. Results of solubility and disintegration:

Results of solubility are shown in table (3). The experimental cement gave a higher disintegration percentage than the commercial cement however, when reported to the nearest 0.1% as stated in the ADA specification No (8), the two results were equal (0.1%).

Table (2): The mean and standard deviation (S.D) of the working time, setting time and film thickness of the commercial and experimental cement.

Properties / Mixture type	L/P ratio (ml/gm)	Mean working time(in minutes)	Mean setting time (in minutes)	Mean film thickness (in µm)
Commercial cement	3:1	5 ±1	7.5 ±0.5	25 ±0.5
Experimental cement	3:1	4 ±0.5	7.5 ±0.2	25 ±0.1

Table (3): The mean disintegration % of the commercial and experimental cement

Table (3): The mean disintegration % of the commercial and experimental cement / Mix type	L/P ratio (in ml/gm)	Disintegration %
Commercial cement	3:1	0.099% ≈ 0.1%
Experimental cement	3:1	0.126% ≈ 0.1%

3.3. Results of compressive strength

Results of compressive strength are shown in table (4). Results revealed that the experimental cement gave a lower compressive strength (42.09 MPa) compared with that specified by the ADA specification No. (96) (70 MPa), however there was no statistical significant difference between the experimental and the commercial zinc phosphate cement (49.6 MPa).

Table (4): The mean and standard deviation of the compressive strength of the commercial and experimental zinc phosphate cements.

Mix type	L/P ratio (in ml/gm)	Compressive strength (in MPa)
Commercial cement	3:1	49.6 ±1.2
Experimental cement	3:1	42.09 ±2.2

4. Discussion

Dental zinc phosphate cements use a special form of zinc oxide that has been deactivated by mixing with magnesium oxide. Silica and alumina are added to reinforce the set cement, followed by being sintered together at temperatures between 1000ºC and 1400ºC then grounded to a fine powder John (2002). According to Safwat et al,(2007)several additives were tried to justify the fluidity, the working and setting time. The additives used were carefully selected in the light of the published literature on similar materials, their availability at local markets and their cheapness. Although, these additives should improve some properties, yet they should not alter or destroy other properties. The bile bovine additive is a yellowish powder obtained after grinding of ox-gall stones. It is used as a wetting agent in marbling paper industry. The bile bovine alters the surface tension of the cement allowing it to spread out over the surfaces. The bile bovine additive promotes the penetration of the particle clumps with water because part of the bile salts present in its composition, is soluble in water Wolfe (1990). The addition of 1.43% bile bovine and 0.9% borax improved working time and setting time of zinc phosphate cement. The added borax influenced the rate of cement hydration resulting in reduction in the setting time and increasing the early strength development. Moreover it disperses cement particles; thus increasing the flow of the experimental cement resembling that of the commercial cement. This may be also due to its adsorption on the particles surfaces leading to a mutual repulsion of individual particles and reduction in interparticles friction, Zaki et al.,(2006). Consequently the combination of borax and bile bovine increase the setting time allowing more particles in the mixture to react.

The mean compressive strength of the commercial cement and experimental cement (49.6 MPa), (42.09 MPa) respectively, did not reach the ADA specification limits (70 MPa). This may be due to the porosity in the set mass resulted from human errors during samples preparation. This result is in agreement with Goto et al (1999) where 25% of the mixed cement had strength values below 40 MPa. They emphasized that the ideal mixing conditions of the cement components are seldom achieved.

From the foregoing discussion, it can be concluded that, the prepared experimental cement properties simulated that of the commercial cement and met the specification requirements except for the compressive strength. Further investigations are required to improve the properties of the experimental cements to reach that of the commercial cement as regards compressive strength.

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Assessment of Egyptian buffaloes crossing with Pakistani and Italian buffaloes for some production traits

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Abstract: Egyptian buffaloes are considered one of the most important dual purpose farm animals that represent 44% of dairy animals in Egypt. In 1980, the Animal Production Research Institute (APRI) imported 93 Pakistani semen straws for crossbreeding to improve milk productivities. In 2003, Ministry of Agriculture (MoA) allowed the commercial importation of Italian buffalo semen, which spread in large scale buffalo farms. The study aims to evaluate the Egyptian buffalo crosses with both Pakistani and Italian buffaloes for some productive traits to assess the crossing trials. For the first trial of the study, 180 records (85 pure Egyptian buffaloes (E), 22 record ½Egyptian (EG)½ Pakistani (PA) buffaloes and 52 record ¾E ¼P buffaloes and 21 record 7/8E 1/8P) through the period from 1980 to 1998 were used for the evaluation of Egyptian (EG) Pakistani (PA) crossbred. Data for the second trial, concerned with the evaluation of the Egyptian (EG) Italian (IT) crosses, was collected from two private farms. A total 138 records; 64 record from Ganat Elreda farm (32 record EG and 32 record ½EG ½IT) and 74 records from "United Group farm" (26 record EG and 48 record ½EG ½IT buffaloes) were utilized. Utilized record covers the period from 2005 to 2009. Average for total milk yield was nearly the same for Egyptian and its cross with Pakistani buffaloes. In trial 1, Milk yield generally tended to increase with the advancement of parities till the ≥7 parity**.** Egyptian buffaloes showed the highest values for all growth traits measures. In trial 2, significant difference in milk productivity between the Egyptian and its Italian crossbred, which was significantly higher ($P \leq 0.001$) in farm 2 than it is in farm 1 ($P \leq 0.01$), was observed. The same trend in difference was detected for the parity effect**.** Italian crosses showed higher least square means (LSM) estimates for total milk yield (TMY) than the Egyptian buffaloes, which also increased with the advancement of the parity, in the two farms. LSM data reveal increase of 27 and 15% in 1/2EG1/2IT crossbred milk production than the Egyptian in farm 1 and farm 2, respectively. Difference between the highest and lowest breeding value (BV) in the Egyptian population is larger than it is in the crossbred population. More studies are recommended for the assessment of productive, reproductive and genetic diversity of crossbred populations before the enhancement of crossbreeding activities on national level.
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Keywords: Egyptian, Pakistani and Italian buffaloes, crossing, production traits, breeding value.

1. Introduction

Egyptian buffaloes are considered one of the most important farm animals that kept for dual purposes (milk/meat production). There are nearly 4 million buffaloes, representing 44% of dairy animals in Egypt (FAOSTAT, 2009), which contribute 44 % (2,640,638 ton) of total milk production (5,960,102 ton) and 18 % (270,000 ton) of total meat production (1,528,789 ton) in Egypt (FAOSTAT, 2008). Egypt suffers from a huge production gap in milk and meat detected in annual imported milk and meat (http://www.fao.org). Production of buffalo in Egypt couldn't fill such a gap due to the absence of specialized breeds/lines for (meat/milk) and the need for national genetic improvement programs.

Therefore trials for the introduction of foreign breeds of buffalo (crossbreeding with both Italian and Pakistani breeds) were performed with the aim to significantly improve the genetic makeup of the Egyptian buffaloes for economic traits, as in case of the native cattle crossbreeding.

Pakistani buffaloes have the potential of producing over than 5,000 liters of milk per lactation under efficient breeding, feeding and health care program. Nili Ravi is the best breed at national and international level in terms of its production potentiality, reflected in average milk yield per lactation of 2,430 liters, while some high yielding Nili Ravi also produce 3000-5000 liters/lactation (Bilal *et al.*, 2006).

In Italy there are 300,000 buffaloes, used to be found in the central and south of Italy. Due to the quota on cattle milk, buffaloes have moved towards the north and replaced a portion of dairy cows. The number of recorded Italian milking buffaloes is around 44,000 (one third of the total buffalo population). Average milk production is 2,250 kg/lactation. It has increased the last 17 years, more

depending on better management than on genetic improvement (Maria Larsson, 2009).

No previous assessment, neither genetic nor phenotypic, has been performed for assessing such crossbreeding. The main objective of the present study is to evaluate the Egyptian buffalo crosses with both Italian and Pakistani buffaloes for some productive traits to assess the crossing trials.

2. Material and Methods

This study is divided into two trials; the first is concerned with the assessment of Egyptian-Pakistani crosses (EGPA), where the animals included belong to experimental herds kept in Mahalet Mousa farm, belonging to Animal Production Research Institute (APRI), Ministry of Agriculture (MoA), Egypt. In 1980, APRI imported 93 Pakistani semen straws for crossbreeding trials for improving milk production of buffalo. APRI practiced a crossbreeding scheme since then with different crossing ratios. A total of 180 records (85 pure Egyptian buffaloes (EG), 22 record ½Egyptian (EG)½ Pakistani (PA) buffaloes and 52 record ¾EG ¼PA buffaloes and 21 record 7/8EG 1/8PA) through the period from 1980 to 1998 were used for the evaluation. Traits included are total milk yield (TMY), lactation period (L), birth weight (BW), weaning weight (WW) and daily gain (DG) calculated for the period from birth to weaning.

The data were analyzed using SAS (2002), according to the following model for total milk yield:

$$\mathbf{Y_{ijk} = \mu + B_i + P_j + b(L)_{ijk} + (BP)_{ij} + (BL)_{ijk} + (PL)_{ijk} + E_{ijk}} \quad \mathbf{(1)}$$

Where: Y_{ijk} : observation on the k^{th} animals of the i^{th} population in the j^{th} parity, μ : overall mean, B_i : fixed effect due to the population, (i: EG, ½EG½PA and ¾EG¼PA), P_j : fixed effect due to lactation parity, (j: 1, 2, …, 6, ≥7), b : regression coefficient of Y on L (lactation period), (BP) : the interaction between breed and parity, (BL) : the interaction between breed and lactation period, (PL) : the interaction between parity and lactation period and E_{ijk} : random error assumed N.I.D. (0, $\sigma^2 e$).

While the model used for birth, weaning weight and daily gain traits was:

$$\mathbf{Y_{ijk} = \mu + B_i + S_j + E_{ijk}} \quad \mathbf{(2)}$$

Where: $Y_{ijk:}$ observation on the k^{th} animals of the i^{th} population in the j^{th} sex of calve, μ: overall mean, B_i: fixed effect due to the population, (i: EG, ½EG ½PA, ¾EG ¼PA and 7/8EG1/8 PA), P_j : fixed effect due to sex of calve, (j: male and female) and E_{ijk} : random error assumed N.I.D. (0, $\sigma^2 e$).

The second trial is concerned with the evaluation of the Egyptian (EG) –Italian (IT) crosses. Two large-scale dairy buffalo farms were included in this study being "Ganat Elreda" farm in Ismaeleia governorate and "United Group" farm in Qaliobeia governorate. The two farms select their Egyptian milking buffaloes from animal markets in their second parity. They keep the new purchased lactating animals under assessment, for production and health conditions, for two weeks, and then they decide to keep or cull them. It seems successful practical selection rules under the conditions of absence of pedigree and production recording system in the majority of small and medium scale buffalo holdings. For crossbreeding, they use imported Italian buffalo semen with known breeding values for various production and type traits. A total 138 records; 64 record from Ganat Elreda farm (32 record EG and 32 record ½EG½IT) and 74 records from "United Group farm" (26 record EG and 48 record ½EG½IT buffaloes). Records covering the period from 2005 to 2009 were used for evaluating the crossbreeding performance for total milk yield (TMY), lactation period (L), birth (BW) and weaning (WW) weights traits.

Data was analyzed according to the following model for total milk yield :

$$\mathbf{Y_{ijkm} = \mu + B_i + P_j + C_k + S_l + b_1(L)_{ijklm} + b_2(A)_{ijklm} + (LA)_{ijklm} + E_{ijklm}} \quad \mathbf{(3)}$$

Where: Y_{ijklm} : observation on the m^{th} animals of the i^{th} population in the j^{th} parity in the k^{th} year of calving in the l^{th} season of calving, μ: Overall mean, B_i: fixed effect due to the population, (i: EG and 1/2EG1/2IT), P_j : fixed effect due to lactation parity, (j: 1, 2, 3), C_k : fixed effect due to the year of calving, (k: 2007, 2008, 2009), S_l: fixed effect due to the season of calving (l: Winter and Summer), b_1: regression coefficient of Y on L (lactation period), b_2: regression coefficient of Y on A (Age at first calving), (LA): the interaction between lactation period and Age at first calving and E_{ijklm}: random error assumed N.I.D. (0, $\sigma^2 e$).

While the model used for birth and weaning weights traits was:

$$\mathbf{Y_{ijkl} = \mu + B_i + C_j + S_k + E_{ijkl}} \quad \mathbf{(4)}$$

Where: Y_{ijk} : observation on the m^{th} animals of the i^{th} population in the j^{th} year of calving in the k^{th} season of calving, μ : Overall mean, B_i: fixed effect due to the population, (i: EG and ½EG½IT), C_j: fixed effect due to the year of calving, (j: 2005, 2006, 2007), S_k: fixed effect due to the season of calving (k: Winter and Summer) and E_{ijkl}: random error assumed N.I.D. (0, $\sigma^2 e$). Only female data was available in the studied farms, therefore, the sex effect was not included in the model.

The animal model (derivative-free restricted maximum likelihood, DFREML, Meyer, 1997) was used for the prediction of buffaloes breeding value for TMY trait according to the following model:

$$\mathbf{Y = Xb + Z_a a + Z_c c + e} \quad \mathbf{(5)}$$

where: Y = Vector of observations, X = Incidence matrix relating fixed effects to y, b = Vector of an overall mean and fixed effects (parity, year, season of calving and lactation period and age at first calving as a covariable), Z_a = Incidence matrix relating direct additive genetic effects to y, a = Vector of random effect (direct additive genetic associated with the incidence matrix Za, Z_c = Incidence matrix for permanent environmental effect, c = Vector of permanent environmental effect associated with the incidence matrix Zc and e = Vector of random residual effects N (0, $I\sigma^2e$); I is an identity matrix. The variance-covariance of the random effects was as follows:

$$Var \begin{bmatrix} a \\ c \\ e \end{bmatrix} = \begin{bmatrix} A\sigma^2a & 0 & 0 \\ 0 & I_c\sigma^2c & 0 \\ 0 & 0 & I_n\sigma^2e \end{bmatrix}$$

Where: A = Numerator relationship matrix, I_c, I_n = Identity matrix with order equal to number of animals and number of records, respectively.

3. Results and discussion

Trial1:

Unadjusted means, standard deviations and number of records for total milk yield and lactation period are presented in Table 1.

Table (1). Unadjusted means, standard deviations (SD) and number of records (No.) for total milk yield (TMY) and lactation period (LP) in Egyptian (EG) and their crossing with Pakistani (PA) buffaloes.

Buffaloes population	TMY (Kg)		LP (Day)		NO.
	Mean	SD	Mean	SD	
Egyptian (EG)	1502	344	263	60	85
1/2EG½ PA	1357	394	218	47	22
3/4EG1/4PA	1383	372	193	42	29

Average total milk yield was nearly the same, considering the lactation period, for Egyptian buffaloes and its crossing with Pakistani, but Egyptian group had lower degree of deviation. For lactation period, Egyptian buffalo showed its ability to persist longer lactation than its two crosses groups. Reviewing other research articles for contemporary herds, averages total milk yield were similar to those reported by Abd El-Raoof (1995) but higher than those reported by Mostageer et al. (1981); Khattab *et al.* (1985); Kotby *et al.* (1989); Khalil *et al.* (1992); Khattab and Mourad (1992); Khalil (1993) and Mansour *et al.* (1993) in Egyptian buffaloes. It was lower than the estimates, 1564, 2159 and 1879 kg, obtained by Soliman *et al.* (1985); Ashmawy (1991) and Mourad and Mohamed (1995), respectively.

Mean-squares estimates for lactation period, shown in Table 2, present significant differences between the three studied populations, and the population/lactation interactions ($P \leq 0.05$). Expectedly, lactation period covariate had highly significant effect on milk yield, while lactation parity did not have significant effect on TMY. This result is in disagreement with Soliman (1976); Kotby *et al.* (1989); Ashmawy (1991); Khalil *et al.* (1992); Khalil (1993) and El-Menshawy (1994) for Egyptian buffaloes, all reporting high significant differences between lactation parities. Ashmawy (1991) reported that the effect of parity on milk yield traits with advance of lactation order may be attributed to the increase in weight, size, advancement in age, and/or developing the udder secretory tissues until reaching full development.

Table (2) Mean squares (MS) for total milk yield.

Source of variation	d.f	MS
Population (B)	2	315609*
Lactation parity (P)	6	117966
Covariable		
Lactation period (L)	1	2690583***
Interactions:		
B*P	12	96076
L*P	6	99301
L*B	2	278976*

*** : P ≤ 0.05** ***** : P ≤ 0.001**

Milk yield generally tend to increase with the advancement of parities till the ≥7 parity group (Table 3). These results are in agreement with Soliman (1976); Khalil *et al.* (1992) and El-Menshawy (1994). Such results reflect the buffalo ability to develop milk production and its biological processes for longer production life span (longevity) than the cattle. The population of ¾EG¼PA showed the highest milk production followed by the ½EG½PA population, reflecting the effect of crossbreeding ratio on the trait. The higher Egyptian blood percentage group was favorable, which might be due to the adaptation of Egyptian buffalo population to the Egyptian environment.

Table (3). Least squares means (LSM) and their standard errors (SE) of factors influencing total milk yield (TMY).

Effect	No.	LSM±SE
Parity :		
1	18	1339±150.24
2	25	1271±119.21
3	28	1552±78.23
4	25	1512±111.12
5	13	1429±117.48
6	11	1585±100.71
≥7	16	1664±88.26
Buffaloes population :		
Egyptian (EG)	85	1441±42.74
1/2 EG 1/2 PA	22	1469±107.97
3/4 EG 1/4 PA	29	1527±97.35

Egyptian buffaloes showed the highest values for all growth traits measures, accompanied with higher deviation (Table 4). Result is in agreement with Fahmy (1972) Mostageer *et al.* (1981) and Alim (1991) for BW, while, lower than Fooda (1996); (42.8-44.9 kg) and El-Menshawy (1994); (42 kg). For WW, the result is in agreement with El-Naggar *et al.* (1972); Fahmy (1972) and Mahdy *et al.* (1999), while is lower than data reported by Mostageer *et al.* (1981) and El-Menshawy (1994). It is higher than Fooda (1996) and Salama and Mohy El-Deen (1997). The result for DG (from BW to WW) is in agreement with Salama and Mohy El-Deen (1997) and Mahdy *et al.* (1999) but is lower than El-Menshawy (1994); (being 0.66 kg).

Table (4) Unadjusted means, standard deviations (SD) and number of records (No.) for birth (BW), Weaning (WW) weights and daily gain (DG) in Egyptian (EG) and their crossing with Pakistani (PA) buffaloes.

Buffaloes Population	BW (Kg)			WW (Kg)			DG (Kg)		
	Mean	SD	No.	Mean	SD	No.	Mean	SD	No.
Egyptian (EG)	35.0	6.4	73	92	8.4	73	0.54	0.06	73
1/2EG1/2PA	34.0	1.5	22	-	-	-	-	-	-
3/4EG1/4PA	34.0	3.7	52	79	4.7	13	0.44	0.03	13
7/8EG1/8PA	32.0	7.3	21	82	3.5	3	0.47	0.06	3

Although significant differences ($P \leq 0.001$) were detected in both weaning weights and daily gains among studied populations, birth weight trait did not show significant differences (Table 5). Birth weights differed significantly between the two sexes ($P \leq 0.01$). This result is in agreement with Sadek (1980) and Tantawy (1984), but disagree with Fooda (1996). Weaning weight and daily gain traits were not significantly affected by sex. This result is in disagreement with Sadek (1980) and Tantawy (1984), which might be due to the farm management practices, in rearing and growth periods that did not challenge males' potentiality for growth.

Table (5) Mean squares (MS) for body weights and daily gain.

Source of variation	BW		WW		DG	
	d.f	MS	d.f	MS	d.f	MS
Population (B)	3	58.656	3	1007.436***	3	0.0576***
Sex (S)	1	189.116**	1	165.137	1	0.0021

**** : $P \leq 0.01$, *** : $P \leq 0.001$**

Egyptian buffaloes showed superiority in growth traits studied (weaning weight and daily gain). Population of 7/8EG 1/8PA showed higher estimates for the same traits than the 3/4EG 1/4PA indicating the effect of Egyptian population (Table 6). Mahdy *et al.* (1999) reported the same result for the DG between BW and WW.

Table (6). Least squares mean (LSM) and their standard errors (SE) for factors influencing birth (BW), Weaning (WW) weights and daily gain (DG).

Effect	Birth weight (BW, Kg)		Weaning weight (WW, Kg)		Daily gain (DG, Kg)	
	No	LSM±SE	No	LSM±SE	No	LSM±SE
Sex :						
Male	16	37±1.01	4	91±4.53	4	0.51±0.03
Female	152	33±0.49	85	83±1.76	85	0.48±0.01
Buffaloes population.:					73	
Egyptian (EG)	73	37±1.01	73	96±2.51	-	0.56±0.02
1/2EG 1/2PA	22	36±1.39	-	-	13	-
3/4EG 1/4PA	52	35±0.80	13	81±2.34	3	0.44±0.02
7/8EG 1/8PA	21	34±1.41	3	86±5.08		0.48±0.04

Trial 2:

For the Egyptian buffalo in both farms, first parity yields were higher than the second, while the third parity, estimated for one farm, was the highest (Table 7). Low productivity in the second parity is due to the purchase of under-test new animals from the animal market in their second parity, according to the farm management. Generally in farm1, crossing buffalo produced more milk than the Egyptian in all parities, while in farm 2 Egyptian buffalo produced more milk than the crossing population at first parity. Egyptian buffalo showed longer lactation period than the crossbred population, indicating higher production persistency, with lower daily milk yield, except the third parity in farm 1. Some crossbred animals showed superior productivities in their second lactation (>4500 kg), which resulted in increase in standard deviation. Authors kept all the available records to avoid biasness. In Italy, TMY and LP for Italian buffaloes (measured for only nationally recorded dairy buffaloes; presents 28% of total population) were 2,175 kg and 270d, respectively (Maria Larsson, 2009). In the same reference, author reported TMY and LP for Egyptian buffaloes as 1,600 kg and 321 d, respectively, therefore, estimated daily milk yield for Italian and Egyptian buffaloes, respectively, are 8 and 5 kg/d. According to Fooda et al. (2010) reported the daily

milk yield to be 8 kg/d/head measured from 3,495 records collected from 904 buffalo cows.

Table (8) shows the significant difference in milk productivity between the Egyptian and its Italian crossbred population, which was significantly higher ($P \leq 0.001$) in farm 2 than it is in farm 1 ($P \leq 0.01$). That might be due to different management decision in selection of the purchased Egyptian buffalo animals in the two farms. The same trend in difference is detected for the parity effect, due to the purchase of new milking animals to join the herd in their second parity, affecting the average dairy production of the farm. Parities in farm 2 do not exceed the second (Table 9).

Insignificant effect of season on milk production indicates the good management system in both farms that compensate the season effect. The sever change in lactation period between the first and the second parity, in farm 2 (Table 7) resulted in the significant effect of lactation period ($P \leq 0.01$) on milk yield (Table 8). Lactation phase of new purchased animals should be considered, to be corrected for. Both covariables, showed significant effect only in farm 2, indicating the variability in both lactation phase and the age of the Egyptian animals at purchase time.

The correction of fixed effects and covariates reveal that the Italian crosses showed higher LSM estimates values for TMY than the Egyptian buffaloes, which also increases with the advancement of the parity, in the two farms (Table 9). LSM data reveal increase of 27 and 15% in 1/2EG1/2IT crossbred milk production than the Egyptian in farm 1 and farm 2, respectively.

Both raw mean and LSM data (Tables 10 and 12) show that Italian crosses are superior to Egyptian buffalo in both body weights (birth and weaning), in both farms. Generally, the Italian buffalo has a body conformation that very likely to meat production animals, in addition to its superiority in milk production due to genetic improvement for dairy production and type traits. Fooda et al. (2009), reported that birth and weaning weights were 33.5 and 77.28 kg for the Egyptian buffalo using the data for 148 females and 96 males, raised in APRI experimental farms.

MSE estimates reveal that the buffalo population (Egyptian vs Italian crossbred) has the significant effect on both studied weights, except the WW in farm 2 (Table 11). For the Egyptian buffaloes kept in APRI experimental farms, Fooda et al. (2009) reported insignificant effect of year and season of calving on birth weight, while, the same author reported highly significant effects on weaning weight, for the same effects. No trend was detected for birth weight during the period covered in the study, while weaning weight showed positive trend of increase.

Figures 1 and 2 illustrate the highest and lowest breeding value for total milk yield in both included farms. Figures (1 and 2) show that the difference between the highest and lowest breeding value (BV) in the Egyptian population is smaller than it is in the crossbred population (+101 to -269; 370 kg, and +425 to -135; 560 kg, for the Egyptian and crossbred populations, respectively) in farm 1, while the opposite situation is noticed in farm 2 (+85 to -181; 266 kg, and +81 to -70; 151 kg, for the Egyptian and crossbred populations, respectively). Comparison between the two farms indicates that farm 1 have more potential animals. Farm 1 milking buffaloes showed much wider range (maximum and minimum) breeding value than Farm 2 (+424.64 vs +85.47 for the maximum, and -269.49 vs -181.16 for the minimum).

Table (7). Unadjusted means, standard deviations (SD) for total milk yield (TMY) and lactation period (L) at various parity (P) in Egyptian (EG) and their crossing with Italian (IT) buffaloes.

Traits	Farm 1+			Farm 2+		
	Mean	SD	N	Mean	SD	N
Parity 1						
Total milk yield (TMY, Kg)						
EG	1827	358	18	1977	510	18
1/2 EG 1/2 IT	2362	611	19	1647	731	40
Lactation period (L, Day)						
EG	277	46	18	281	65	18
1/2 EG 1/2 IT	265	30	19	209	103	40
Parity 2						
Total milk yield (TMY, Kg)						
EG	1540	363	7	1111	559	8
1/2 EG 1/2 IT	2500	1251	11	2003	900	8
Lactation period (L, Day)						
EG	271	69	7	102	54	8
1/2 EG 1/2 IT	255	46	11	166	78	8
Parity 3						
Total milk yield (TMY, Kg)						
EG	2533	244	9	-	-	-
1/2 EG 1/2 IT	3289	692	4	-	-	-
Lactation period (L, Day)						
EG	273	30	9	-	-	-
1/2 EG 1/2 IT	285	30	4	-	-	-

+ Farm 1: Ganat Elreda; Farm 2: United Group

Table (8) Mean squares (MS) for total milk yield (TMY).

Source of variation	Farm 1+		Farm 2+	
	d.f	MS	d.f	MS
Population (B)	1	3055097**	1	789707***
Lactation parity (P)	2	1438118**	1	2461196***
Year of calving (C)	2	1142014*	1	44963
Season of calving (S)	1	26951	1	144730
Covariable				
Lactation period (L)	1	958218	1	366262**
Age at first calving (A)	1	327317	1	242623*
Interactions:				
L*A	1	316853	1	75239

+ Farm 1: Ganat Elreda; Farm 2: United Group

*** : $P \leq 0.05$ ** : $P \leq 0.01$ *** : $P \leq 0.001$**

Table (9) Least square means (LSM) and their standard errors (SE) for total milk yield (TMY).

Effect	Farm 1+			Farm 2+		
	LSM	SE	N	LSM	SE	N
Population						
EG	2156	135	32	1802	69	26
1/2EG1/2IT	2728	112	32	2077	77	48
Parity						
1	2139	100	36	1524	46	58
2	2338	148	17	2356	120	16
3	2848	191	11	-	-	-
Year						
2007	2790	193	11	-	-	-
2008	2215	119	31	1991	213	20
2009	2320	123	22	1888	48	54
Season						
Winter	2465	117	31	1880	62	45
Summer	2418	116	33	1999	83	29

+ Farm 1: Ganat Elreda; Farm 2: United Group

Table (10). Unadjusted means, standard deviations (SD) for birth and weaning weights (kg) in Egyptian (EG) and their crossing with Italian (IT) buffaloes.

Traits	Egyptian buffaloes (EG)			1/2 EG 1/2 IT		
	Mean	SD	N	Mean	SD	N
Farm 1+						
Birth weight (BW)	35	9.12	6	41	2.15	12
Weaning weight (WW)	91	4.31	6	101	6.86	11
Farm 2+						
Birth weight (BW)	41	4.63	25	46	6.24	49
Weaning weight (WW)	105	4.42	25	106	3.74	49

+ Farm 1: Ganat Elreda; Farm 2: United Group

Table (11) Mean squares (MS) for birth and weaning weights

Source of variation	BW		WW	
	d.f	MS	d.f	MS
Farm 1+				
Population (B)	1	150.358*	1	222.907*
Year of calving (C)	2	15.752	2	83.574
Season of calving (S)	1	14.405	1	30.804
Farm 2+				
Population (B)	1	166.45*	1	6.18
Year of calving (C)	1	6.28	1	7.38
Season of calving (S)	1	60.06	1	1.24

+ Farm 1: Ganat Elreda; Farm 2: United Group
*** : P ≤ 0.05**

Table (12) Least square means (LSM) and their standard errors (SE) for birth (BW) and weaning (WW) weights.

Effect	BW			WW		
	LSM	SE	N	LSM	SE	N
Farm 1+						
Population						
EG	34	2.88	6	92	2.90	6
1/2EG1/2IT	41	1.67	12	101	1.73	11
Y						
2005	39	2.33	7	98	2.62	6
2006	38	2.70	5	92	2.71	5
2007	36	2.53	6	100	2.54	6
SE						
Winter	39	1.79	11	95	1.80	11
Summer	36	2.77	7	98	2.94	6
Farm 2+						
Population						
EG	43	2.50	25	104	1.74	25
1/2EG1/2IT	47	2.01	49	105	1.40	49
Y						
2006	44	0.82	55	106	0.57	55
2007	44	1.55	19	106	1.08	19
SE						
EG	46	2.38	35	105	1.66	35
1/2EG1/2IT	44	2.11	39	104	1.47	39

+ Farm 1: Ganat Elreda; Farm 2: United Group

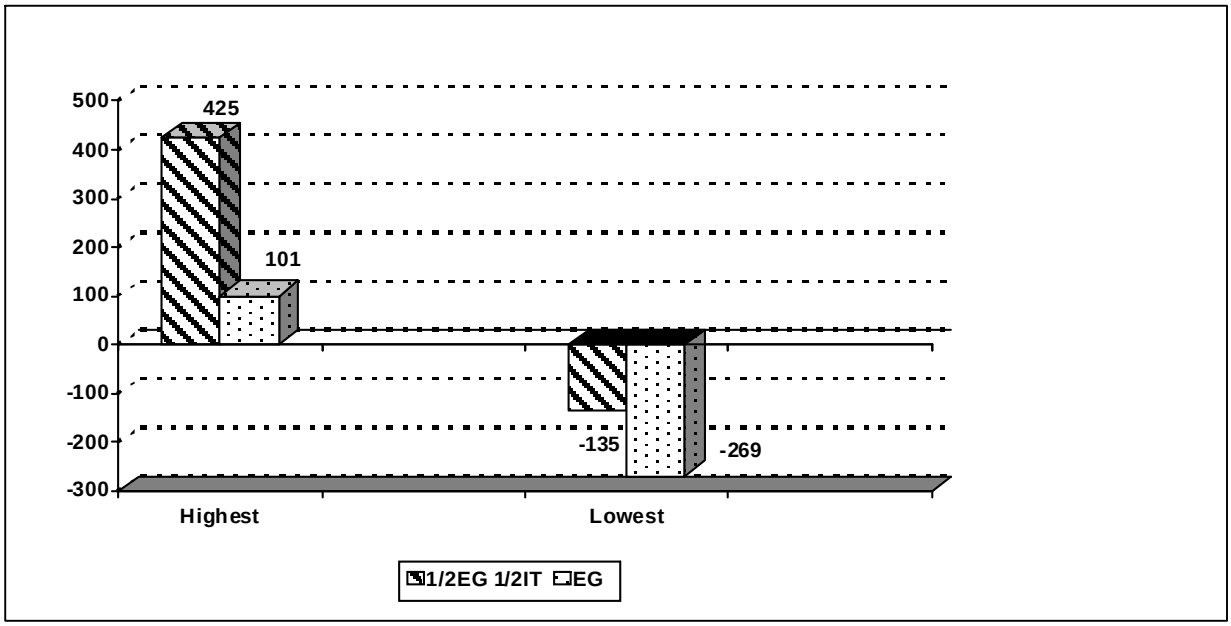

Fig (1). Highest and lowest breeding values for total milk yield in Ganat Elreda farm.

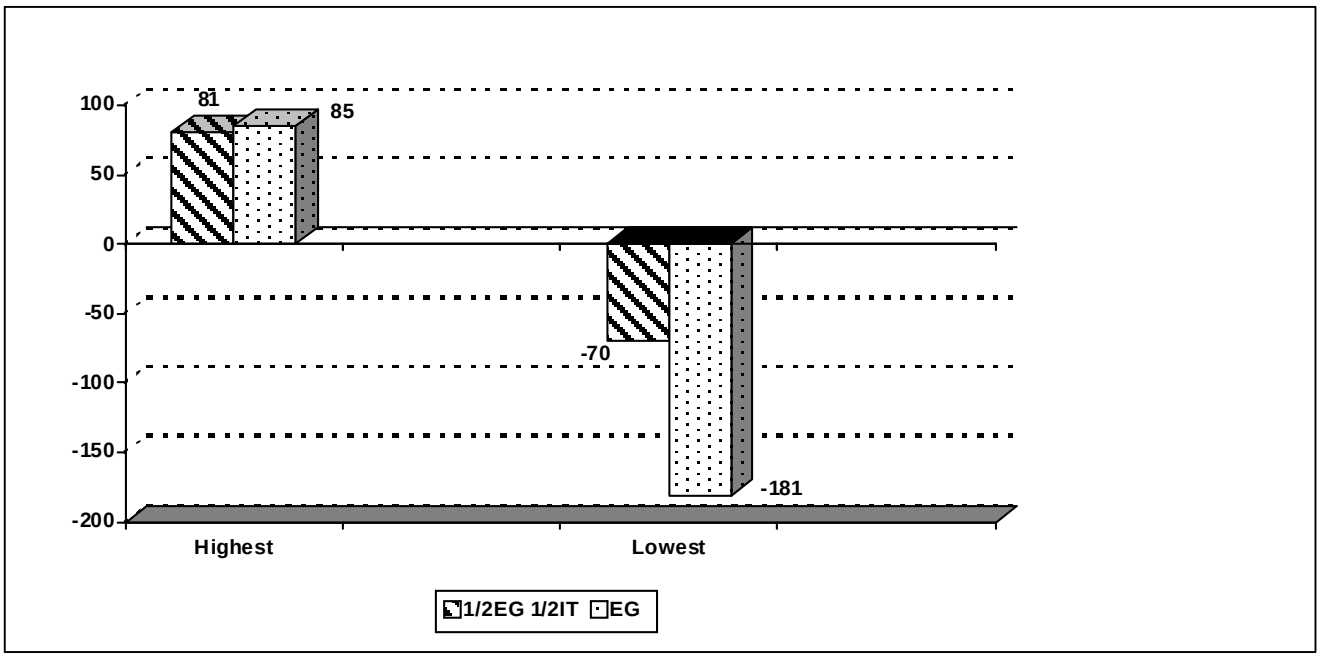

Fig (2). Highest and lowest breeding values for total milk yield in United Group farm.

Conclusion

It can be concluded that from the results obtained:

Trial 1:

- Only slight increase in milk productivity was noticed due to crossbreeding with the Pakistani buffaloes. Concerning weaning weight and daily gain (from birth to weaning), high significant differences were detected, favoring the Egyptian buffaloes population. It can be then concluded that the Egyptian buffalo is a better dual purpose animal than the Pakistani crossbred.

Trial 2:

- Since the Italian buffalo semen is from the same source and with very close breeding values, the difference in crossbred production performance is due to the Egyptian buffalo's genetic merit. Paying more attention to the genetic improvement of the Egyptian buffalo is quite likely to improve its productive performance.
- Reviewing the results obtained for birth and weaning weights raise the question of the opportunities of using Italian buffalo for improving the national meat production from buffalo.
- There is still need for milk composition analysis, lactation curve fitting, and the assessment of the reproductive performance for the crossbred populations for more accurate assessment of crossbreeding and its performance under local conditions.

A general conclusion can be summarizes as more studies are needed for the assessment of productive, reproductive and genetic diversity of crossbred populations before the enhancement of crossbreeding activities on national level.

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Effect of Early versus Late removal of Urinary Catheter on Urinary Outcome after Hysterectomy

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Abstract: Aim of the study: this study aims to explore the effect of early versus late removal of urinary catheter on urinary outcome after hysterectomy. Setting: This study was conducted in the gynecology department of Mansoura University Hospital. Study Design: quasi experimental design. Sample Type:-purposive sample. The study comprised of 100 gynecologic women, they were chosen according to the following criteria:-Complained from symptoms of uterine prolapse, undergoing hysterectomy, their age ranged from 40 ->60 years old and free from any other gynecological problems. They were categorized into two groups: 1) early group, had early removal of urinary catheter 12 – 24 hours after surgery. 2) late group had late removal of urinary catheter after surgery by 48 – 72 hr,s. Results: Urinary symptoms " retention of urine, frequency, burning micturation and UTI were significantly higher in late urinary catheter elimination group as compared to early removal group . Conclusion: Short duration of postoperative catheterization "12-24" hour's is preferred than long duration in which it lead to less urinary problems. Also age of women, degree and duration of uterine prolapse don't play a major role in development of post catheter removal urinary symptoms. Pre existing of postoperative UTI had a main role in the development of these symptoms. Thus it was recommended that ideal time of removal of urinary catheter is from 12-24 hour hysterectomy.

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Key Words: Urinary Catheter, Urinary Outcome, hysterectomy, pyelonephritis, Postoperative.

1. Introduction:

Urinary catheterization as a priority area for the development of a best practice statement, because of the risks of urinary tract infection (UTI) associated with catheterization. The risk of UTI is affected by a number of factors, including the method and duration of catheterization, the quality of catheter care and host susceptibility. Each hospital-acquired UTI results in an increased length of stay of 5-6 days in hospital and has additional cost implications for treatments (Penny, B. ; Chris, H 2005).

Hysterectomy for non-malignant condition is the most common major gynecological surgery performed in women less than the age of 50 years.. Griffiths et al, (2004) Postoperative urinary retention due to bladder over distention and atony, pain, infection, hematoma and the inflated balloon of the standard folly's catheter, all contributes to delayed return of normal micturation. For preventing increased risk of postoperative Urinary retention due to bladder over distention and atony, most gynecologists use urinary catheterization as bladder drainage in women undergoing hysterectomy (Schiotz, H & Tanbo, T . 2006).

Urinary catheter, although it's a necessary procedure, it has certain complication such as increasing the rate of both symptomatic and asymptomatic urinary tract infections, discomfort and pain. Urinary tract infection as the most important complication of catheterization may lead to many serious and unpleasant outcomes such as pyelonephritis, and septicemia, shock and death (Alessandri et al. 2006). Also the risk of urinary tract infection rises with increasing duration of catheterization, a reduction in catheter time might be expected to reduce this risk (Keung, K& Chung, K .2005).

Different gynecological departments at different hospitals have different protocols for post-operative hysterectomy, bladder catheterization, some recommends 48 – 72 hours catheterization, some 3-5 days. Few studies reported that, short duration (24 – 48) post vaginal hysterectomy catheterization is equally effective and has fewer incidences of urinary infection and bladder function returns earlier ,meanwhile the risk of UTI rises with increasing duration (48- 72) of catheterization, so a reduction in catheter time might be expected to reduce this risk (Schiotz, H & Tanbo, T . 2006).

This study was designed to determine the effect of early versus late removal of urinary catheter on postoperative complications. Such as urinary tract infection, voiding disturbance and hospital stay in gynecologic surgeries.

Significance of the study:

From clinical observation many women suffered from discomfort of catheter and urinary tract infection after hysterectomy. Some studies reported that women undergoing hysterectomy had urinary tract infections. Together with the fact that there is no

protocol for the timing of removal of urinary catheter after hysterectomy at Mansoura university hospital

Research Questions:-

1. What is the ideal time of urinary catheter removal after hysterectomy?
2. Does early removal of urinary catheter will reduce urinary tract infection?

2. Materials and Methods:-

Setting: This study was conducted in the gynecology department of EL - Mansoura university hospital.

Study Design: quasi experimental design was used to conduct this study

Type of Sample:- purposive sample

Subjects:-

The study comprised of 100 volunteers gynecologic women. They were chosen according to the following criteria:-

- Complained from symptoms of uterine prolapse.
- Undergoing hysterectomy
- Their age ranged from 40 ->60 years old
- Free from any other gynecological problems.

Subjects were categorized into two groups

Group (1) : - had early removal of urinary catheter (12 – 24 hrs) after hysterectomy.

Group (2):- had late removal of urinary catheter after surgery by 24hr,s or more.

Tools: - two tools were developed and used to collect the necessary data

1- A structured interviewing questionnaire sheet was designed by researcher to include:-

a) Socio – demographic data as; age, education….etc.

b) Question related to gynecological history:
   1. Surgery indication as uterine prolapse and its degrees.
   2. Duration of surgery.
   3. Analgesics used for pain relieve.

c) Observation check list was used to assess complications of the urinary catheter as:
   1. According to Campbell et. al (1995)degree of urinary discomfort “by using visual analogue scale”.
   2. Characteristics of first voiding and its interval and amount.
   3. Occurrence of urinary tract infection (from symptoms encountered).
   4. Ambulation and hospital stay.
   5.

2) Investigation record: a sample of urine was obtained for culture in order to confirm diagnosis of infection,(before & after hysterectomy).

Procedures:-

- This study was conducted during the period started from April (2oo4) to end of March (2005), 11months for data collection.
- -Subjects were categorized into two groups as previous mentioned.
- Every woman in the two groups was assessed individually by using the previous mentioned tools.
- Every woman was followed from the first day before surgery untill the time of hospital discharge.
- Woman approval to participate in the study was obtained.
- Approval of the hospital director was obtained.
- All women in the study had the right to withdraw at any time during the study period.
- The purpose of the study was explained to each woman and informed that data will be kept in secret, this will reflect on increasing their confidence and cooperation.

Pilot study:-

It was carried out on 10 patients to evaluate the applicability and clarity of the tools and assessment of feasibility of implementing the study.

The sample of women included in the pilot study was excluded from the study sample.

Limitation of the study: Some women refused to participate in the study.

3. Results:

Table 1 reveals that, insignificant differences were observed among two groups regarding their age and educational level. The mean age is similar among women with early and late removal of urinary catheter also the same table shows that, most of women in two groups were illiterate.

Table 2 shows that, slightly less than half of women had a first degree prolapse. Insignificant difference was observed among early and late groups regarding analgesic use.

Table 3 reveals that, significant improvements were observed in early removal of urinary catheter group than late removal group regarding urinary discomfort, first voiding disturbance.

Table 4 shows that significant improvement was observed in early removal of urinary catheter group than late removal group regarding first voiding.

The rate of long interval voiding was higher in late urinary catheter removal.

Table 5 reveals that the mean score of hospital stay was higher among late urinary catheter removal group than early group.

Table 1. Characteristics of the Study Sample

Items	(n = 100)			
	Early (n=50)		Late (n=50)	
	NO.	%	NO.	%
*Age(years)				
40-	15	30.0	13	26.0
50-	24	48.0	27	54.0
>60	11	22.0	10	20.0
X + SD	54.2+7.2		54.4+6.8	
*Education				
Illiterate	21	42.0	23	46.0
Primary school	12	24.0	10	20.0
Secondary school	13	26.0	11	22.0
University	4	8.0	6	12.0

Table 2. Gynecological history of the studied women

Items	(n= 100)			
	Early (n=50)		Late (n=50)	
	NO.	%	NO.	%
*Surgery Indication:				
-Prolapse				
Grade 1	24	48.0	21	42.0
Grade2	14	28.0	16	32.0
Grade3	12	24.0	13	26.0
*Duration of operational surgery:	16	32.0	15	30.0
<30min.	28	56.0	30	60.0
30 – 45 min.	6	12.0	5	10.0
>45 min.				
X + SD	98.7+10.6		37.7+9.4	
*Analgesic	6	12.0	4	8.0

Table 3. Urinary Outcome among women in the Study sample

Items	(n = 100)			
	Early (n=50)		Late (n=50)	
	NO.	%	NO.	%
*Urinary discomfort:				
Mild	37	74.0	12	24.0
Moderate	13	26.0	27	5.0
Sever	0	0	11	22.0
*First voiding:				
Disturbance	3	6.0	31	62.0
*Positive result of urinary tract infection:	3	6.0	12	24.0

Table 4. Characteristics of First Voiding urine among women in the Study sample

Items	n = 100)			
	Early (n=50)		Late (n=50)	
	NO.	%	NO.	%
*Interval of voiding:				
• 2-3h.	33	66.0	13	26.0
• 5-8h.	16	32.0	29	58.0
• 9-11h.	1	2.0	8	16.0
*Spontaneously:	48	96.0	30	60.0
• Bed pan	10	20.0	19	38.0
• Bathroom	40	80.0	11	22.0
*Interrupted				

Table 5. Ambulation and Hospital Stay among women in the Study sample

Items	(n= 100)			
	Early (n=50)		Late (n=50)	
	NO.	%	NO.	%
*Ambulation/h. :				
• 4-	37	74.0	3	6.0
• 9-	10	20.0	10	30.0
• 4-18	3	30.0	37	94.0
*Hospital Stay/h. :				
24	47	94.0	8	16.0
40	3	6.0	33	66.0
>48	-	-	9	18.0

4. Discussion:

This study aims to explore the effect of early versus late removal of urinary catheter on urinary outcome after hysterectomy. This study involved two groups each of them composed of 50 women undergoing hysterectomy due to genital prolapse. First group had early removal of urinary catheter from 12-24 hour after operation; Second group had late removal of urinary catheter from 48-72 hour.

As regarding the women age, the two groups were matched for age ($p > 0.05$), this factor can cause of augment genital prolapse which lead to hysterectomy ,and thus, might give wrong results which affecting on study result . So, the matching criteria to avoid these possibilities. Also women age have a large effect on the incidence of hysterectomy. Also (Dunn, T& Lipsky, B. 2001) added that, hysterectomy for nonmalignant condition is the most common major gynecological surgery performed in women less than the age of 50 year. This finding was similar to the result of the present study in which the mean age was 47 year for women with hysterectomy.

Concerning using of urinary catheter after hysterectomy. In the present study the urinary catheter was inserted after operation for all women in the study sample. This result was explained by Keung,

K& Chung, K .2005 who mentioned that using of urinary catheter after major uncomplicated gynecologic surgery has been the standard method of practice for bladder treatment after operation. Also Phipps et al, (2007) added that urinary catheter was inserted to assess urinary output, improve exposure at the time of surgery and reduce the possibility of injury to urinary tract during surgery.

The urinary catheter was not removed after the operation to prevent postoperative urinary retention and bladder dysfunction however, according to the results of the study; hospital stay, incidence of UTI, ambulation time and level of discomfort were significantly lower in the early catheter removal group. The results provide more evidence (Dunn, T& Lipsky, B.(2001), Getliffe, K& Newton, T.(2006) reported that short duration of urinary catheter after surgery was safe and overall postoperative urinary problems (symptoms , URI) were reduced significantly similarly Fernandez et al, (2003) and Ghoreish, J. (2003)_reported that long duration of urinary catheter was associated with a significantly higher incidence of postoperative urinary retention which might have implications for long term bladder function. Early removal of urinary catheter in stable patients prevents certain complications such as UTI, promotes early ambulation and reduces hospital stay.

As well as Keung, K& Chung, K .2005 mentioned that, Patients with UTI may need additional diagnostic tests and extra medication; this is time consuming because it may increase hospital stay and involve excessive costs. Earlier ambulation may reduce major postoperative complications such as phlebitis and thromboembolism. In addition, length of hospitalization early catheter removal group was significantly shorter than in late group. Therefore, it may decrease complication such as hospital infection and reduce hospital costs.

Also the present study reveals that only a few of women in early catheter removal group required re-catheterization after failing to void and all were able to resume normal voiding , as compared to.. Griffiths et al, (2004) of women in late group.

5. Conclusions and Recommendations

The early removal of in-dwelling catheters after operation was not associated with adverse events as: an increased rate of, urinary tract infections, or need for re-catheterization. In addition, pain assessment was significantly less in the early removal group. As well as short duration of postoperative catheterization “12-24" hour's is preferred than long duration in which it lead to less urinary problems. Also age of women, degree and duration of uterine prolapse don't play a major role in development of post catheter removal urinary symptoms. Pre existing of postoperative UTI have main role in the development of these symptoms. Thus we recommend that ideal time of removal of urinary catheter is from 12-24 hour after gynecological operations.

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Effect of protein feeding system on the quality of milk and its resultant Domiati Cheese

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Abstract: The use of Sunflower meal (SFM) and Leucaena leaves (LL) as a source of 30% of protein requirements in the feeding system of dairy buffaloes and its effect on the yield and composition of milk as well as its resultant Domiati cheese was investigated. The yield of fresh cheese was determined and cheese was pickled in salted whey for 4 months. Samples were taken from milk and also from cheese monthly during storage and were analyzed for moisture, fat, lactose, acidity, amino acids and nitrogen fractions. Formol & Schilovich ripening indices and total volatile fatty acids contents of cheese were estimated as well as their organoleptic properties.Using of SFM and LL increased total solids, fat and total protein of milk. However, the mean values of ash content of milk were lower for SFM and LL treatments. LL milk of LL was the highest in the essential amino acids.Satisfactory of fresh cheese yield (32.12%) for LL treatment, which was higher than control (30.25%) and SFM treatment (30.12%).No significant differences were found among all treatments for the gross composition. Domiati cheese made with LL milk showed the highest total nitrogen and the lowest acidity at the end of ripening period SN/TN % was higher with LL during ripening than SMF and control, while TVFA was higher with control than LL and SFM treatments. Ripening indices FRI & SRI shows that the LL ranged the higher values, followed by that made with SFM and control treatments. The total evaluation scores of fresh cheese were almost the same for all treatments. However, Domiati cheese from LL higher scores than control and SFM at the end of storage period. It can be concluded that sunflower meal and Leucaena leaves can be use as a source of 30% of protein requirements in the feeding system of dairy buffaloes and the milk yielded from this buffaloes can be successfully used in the manufacture of more quality of Domiati cheese.

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Key Words: Domiati cheese system, Sunflower meal ,Leucaena leaves

1. Introduction:

To solve the problem of shortage of milk production, many producers applied various feed-additives to enhance milk yield for providing significant economic income.

Recent development of new feeding system of dairy animals to express protein requirements has placed emphasis not only the protein content of diet but also the protein quality (Satter and Roffer, 1975). Thus, high quality feed proteins including rich amino acids profile and availability of the undegraded protein (Erasmus et al., 1994) may be utilized more efficiently for milk production if large proportions of proteins are less degradable in the rumen.

Sunflower meal (SFM) is a by-product of edible oil industry. It is a rich source of vegetable protein and other nutrient with crude protein (CP): 30.51, ether extract (EE): 0.41, crude fiber (CF): 18.51 and ash: 10.21%. The use of SFM in the ration of lactating cow had a positive effect on solids not fat (SNF) and total solids (TS) of milk, also the cost of milk production was 26.7% less than traditional ration (Jabbar et al., 2008).

Leucaena is a fast growing leguminous tree species that is native to Central America, Mexico and USA. Since its discovery, Leucaena has been distributed throughout many tropical and subtropical areas of the world such as Africa, Australia and India. The CP range for Leucaena is generally accepted to be 20-30% (Gregory, 1996). Leucaena has considerable potential for incorporation into feeding system for dairy cows in the tropics and subtropics. The average increase in milk production obtained from feeding Leucaena was 14% (range of 2-33%). Where recorded, there was usually an increase in fat and protein (Hassan et al., 1989).

Domiati cheese is a soft white salty cheese made primarily in Egypt. It is typically made from buffalo milk, cow milk or mixture milk. It is the most common Egyptian cheese. The yield and composition of cheese vary according to the kind of milk used in cheese making (Abd-El-Aziz et al., 2007).

So, the objective of this study was to evaluate the influence of SFM and LL as a protein source on the yield and composition of milk as well as its resultant Domiati cheese.

2. Materials and Methods:

Animal and rations:

Nine lactating buffaloes in the early lactating season (after 7 days of calving) were used. The animals were randomly assigned into 3 groups of 3 animals each using Latin square design.

Three experimental rations were used in this study. The control diet used (T1) consists of Roughage: concentrate ratio was 30.7% where rice straw composed the basal source of roughage with adding an amount of berseem to adjust crude protein (CP) in the total ration. Sunflower (SFM) meal (T2) and Leucaena (LL) leaves (T3) were used to substitute 30% of the total protein of the concentrate feed mixture (CFM).

The chemical composition of ingredient and rations are shown in Table (1). Animals were feeds twice daily at 6.00 and 16.00 hr. The offered feeds were assesses to cover the requirements for each animal (Kholif and Abd EL-Gawad, 2001).

Analysis of feed samples:

Samples of ingredients and rations were analyzed for Dry matter, ash, crude fiber (CF) CP, Organic matter, ether extract (EE) and nitrogen-free extract, (A.O.A.C, 1995).

Sampling and analysis of milk:

The animals were machine milked twice daily. Milk yield was recorded once every week during the experimental period (90 days, 30 days for each treatment).

At the last 3 days of each experimental period, composite samples were collected from each animal and analyzed for fat, total solids, total protein, non protein nitrogen, acidity and total ash contents were determined as described in IDF standard (1986) and lactose content (Barentte and Abd EL-Tawab, 1957).

Amino acids analysis:

Amino acids composition of milk and cheese samples were determined according to method of Millipore Cooperative (1987) using high pressure liquid chromatographic analysis (HPLC) of amino acids in food using a modification of the PICO-TAG method.

Cheese manufacture

Commercial salt (7%) was added milks and well stirred until dissolved; all treatments were pasteurized at 72°C for 1 min, cooled rapidly to 42°C. Calf rennet (Christian Hansen, Copenhagen, Denmark) was added at 0.1 g/l milk to complete coagulation in 2-3 hr. The curd was scooped into plastic frames lined with cheesecloth and placed over a drainage table and allows draining for 3-4 hr. and pressed to achieve complete drainage in 24 hr. The cheese was cut into blocks and packed in plastic container (500 g), filled with salted whey drained from the same cheese, (EL-Sheikh et al, 2001).

The cheese was stored at room temperature (20 ± 5°C) for 4 months and sampled when fresh and after 1, 2, 3, and 4 months respectively.

Cheese analysis

The samples were first evaluated for their organoleptic properties, homogenized and then stored in the deep freezer until analyze for chemical composition.

Cheese samples were analyzed for moisture, fat and salt as given by A.O.A.C. (1990) and total nitrogen and soluble nitrogen as described in IDF standard (1986). Lactose was determined as described in method of Barnett and Abdel Tawab (1957). Also, Formol and Schilovich (FRI & SRI) ripening indices of cheese were measured according to (Tawab and Hofi, 1966), total volatile fatty acids (TVFA) as described by (Kosikowski, 1978).

The yield of cheese was calculated as kg of fresh cheese per 100 kg of milk, EL-Sheikh et al., (2001). The corrected yield on the basis of 60% moisture was calculated as follows:

Corrected yield = 60% ÷ fresh moisture x calculated yield

Taste panel of 8 persons for National Research Centre (NRC) staff evaluated the organoleptic properties of cheese samples. The panelists scored the cheese for flavour (out of 60 points), body & texture (30 points) and appearance (10 points).

Statistical analysis:

Data obtained from this study were statistically analyzed according to procedures measured by Snedecor and Cochran (1982), Duncan's Multiple Range Test (1955) was used for testing the significant differences between means.

3. Results and Discussion:

Composition of rations:

Table (1), shows the chemical composition of the three experimental treatments. No clear differences between treatments for crude protein (CP), dry and organic matter. Crude fiber (CF) was less when we use LL while nitrogen free extract was higher than control and SFM.

The precentage of the removal of aromatics content is 50%, of sulfur content is 63wt% and nitrogen content is 80.48%.The Refining process decreases mostly the aromatic hydrocarbons in the form of di-and polycyclic aromatics while the monocyclic aromatics are not affected to a big extent as given in Table (1). It is observed that the polycyclic aromatics are completely removed. The refining process removes 80% of dicyclic aromatics.

Table (1): Chemical composition of the three experimental rations (% on dry matter basis).

Item	(T1)	(T2)	(T3)
Dry matter	90.13	90.33	90.21
Organic matter	89.87	90.44	90.82
Crude protein (CP)	13.72	13.53	13.57
Crude fiber (CF)	20.26	18.22	15.24
Ether extract EE)	3.78	3.22	3.52
Nitrogen free extract	52.11	55.47	58.49
Ash	10.13	9.56	9.18

T1 = Control
T2 = Sunflower meal (30%)
T3 = Leucaena (30%)

Milk yield and composition:

The use of Sunflower (SFM) meal (T2) and Leucaena (LL) leaves (T3) increased milk and 4% fat corrected milk (FCM) yield ($P > 0.05$, $P < 0.05$) than control (T1). The use of LL had a relative improvement in milk production, this may be attributed to leucaena had a lower nitrogen solubility than concentrate feed mixture (CFM) and SFM. Similar results were obtained by Grummer and Clark (1982), Khattab et al., (1998). Moreover, LL contains tannins which may be important in the protection of protein from degradation, Jones (1979).

Data of milk composition (Table2) indicated that averages of milk fat, TS, SNF and total protein (TP) were insignificant higher ($P > 0.05$) in LL than those of control and SFM, in addition lactose content of milk was increased significantly ($P < 0.01$) in LL than those of control and SFM. However the mean values of ash content of milk was lower for SFM and LL than that of control. Non-protein nitrogen (NPN) content of milk showed insignificant differences among treatments. The mean values of milk acidity were higher in the evening than that in the morning milking.

Table (2): Composition of buffaloes milk from different treatment used in Domiati cheese manufacture.

Item	T1	T. 2	T. 3	SD
Milk yield kg/day	7.62	8.24	8.58	± 1.75
Total solids	16.00	16.18	16.53	± 0.22
Fat corrected milk	10.34^{bc}	11.26^{ac}	11.77^{a}	± 2.05
Fat	6.30	6.41	6.67	± 0.16
Solids not fat	9.70	9.77	9.86	± 0.007
Total protein	4.00	4.31	4.46	± 0.19
Non protein nitrogen	0.039	0.035	0.033	± 2.49
Ash	0.85	0.77	0.72	± 0.05
Lactose	4.36^{b}	4.40^{b}	4.78^{a}	± 0.19
Acidity morning	0180	0.189	0.186	± 0.004
evening	0.179	0.191	0.187	± 0.005

T1 = Control
T2 = Sunflower meal (30%)
T3 = Leucaena (30%)

- Means of 3 samples for each treatment
- Significant different, according to Duncan's ($P \leq 0.05$)
- A, b and c or ($P < 0.01$) between means are indicated by dissimilar superscripts.

Amino acids content of milk:

Data in Table (3) showed that the milk of buffaloes fed CFM (T1) contained the lowest concentration of most essential amino acids. MILK OF (T3) was the highest in histidine, lysine, methionine and phenylalanine, while (T2) was the highest in arginine and threonine. The control (T1) was the highest in leucine, isoleucine and valine. In addition, data of Table (3) showed that the total essential amino acids of milk were higher in T3 (58.67) g/100 g milk protein than those of T1 (49.72) g/100 g milk protein and T2 (57.15) g/100 g milk protein. This may be due to the lower degradation of protein of leucaena treatment.

Data in Table (3) showed that T2 and T3 recorded nearly similar values of total non-essential amino acids and were lower than T1. Same results were obtained by Khattab et al., (1998).

Table (3): Effect of protein source in rations of lactating buffaloes on amino acids composition of milk (g/100 g milk protein).

Amino acids	T1	T. 2	T. 3	SD
Essential				
Arg	4.68	10.08	8.51	± 2.27
His	1.06	1.31	1.65	± 0.24
Ile	8.09	6.31	5.97	± 0.93
Leu	8.83	6.71	6.89	± 0.96
Lys	8.23	14.75	15.47	± 3.26
Met	2.18	2.29	3.92	± 0.79
Phe	5.65	6.10	6.93	± 0.53
Thr	3.59	4.68	6.86	± 1.2
Val	7.41	4.92	5.47	± 1.07
Total essential	49.72	57.15	58.67	
Non-essential				
Ala	1.66	1.55	1.31	± 0.15
Asp	7.27	6.87	6.72	± 0.23
Cys	1.93	2.30	1.86	± 0.19
Glu	13.03	13.66	13.74	± 0.32
Gly	1.99	2.24	2.17	± 0.11
Pro	1.27	1.63	1.86	± 0.24
Ser	2.27	1.95	1.71	± 0.23
Tyr	10.51	6.75	6.22	± 1.91
Total non-essential	39.93	36.92	35.59	

T1 = Control
T2 = Sunflower meal (30%)
T3 = Leucaena (30%)

Cheese yield:

Table (4): Yield of Domiati cheese made from different treatments.

	T1	T2	T3
Fresh yield	30.25	30.46	32.12
Corrected yield	29.67	29.93	32.73

T1 = Control
T2 = Sunflower meal (30%)
T3 = Leucaena (30%)

Table (4), shows the fresh and calculated yield based on 60% moisture of Domiati cheese from different treatments. LL cheese gave the highest fresh and corrected yield than SFM and control treatments.

It is shown in Table (5), that the moisture content of Domiati cheese decreased gradually as ripening progresses in all treatments. Similar results were reported by EL-Sheikh et al., (2001).

Also, data in Table (5) shows that the F/DM values gradually increase with increasing ripening period. However, T3 showed the highest value (52.32%) at the end of ripening, same trend were also noted for total nitrogen content. However, control cheese showed lowest total nitrogen content (2.54%) at the end of ripening. Salt/DM % showed gradually decreased for all treatments during ripening, T3 showed lowest value (10.03%) at the end of ripening period.

The acidity of cheese increased gradually for all treatments and the cheese resultant from T3 showed the lowest acidity (1.90%) at the end of ripening period.

Data in table (6) showed that T3 contained the highest concentration of essential amino acids (416.81 mg/100g) at the end of ripening period.

Gross composition of cheese:

Table (5) : Gross composition of Domiati cheese from different treatments.

	Moisture %	F/DM %	T. N %	Salt/DM %	Acidity %
Fresh	61.17	40.68	2.21	14.19	0.330
T1	± 0.372	± 0.676	± 0.046	± 0.306	± 0.009
	61.07	44.85	2.36	13.59	0.347
T2	± 0.463	± 0.606	± 0.030	± 0.265	± 0.014
	58.88	44.32	2.42	12.50	0.273
T3	± 0.462	± 0.232	± 0.019	± 0.165	± 0.019
1 month	59.75	42.90	2.31	12.42	0.808
T1	± 0.207	± 0.672	± 0.035	± 0.236	± 0.022
	59.20	46.70	2.49	11.91	0.817
T2	± 0.456	± 0.405	± 031	± 0.067	± 0.023
	57.45	46.26	2.55	11.04	0.735
T3	± 0.302	± 0.163	± 0.015	± 0.070	± 0.021
2 months	58.42	45.68	2.40	11.90	1.23
T1	± 0.306	± 0.319	± 0.095	± 0.119	± 0.032
	57.65	48.26	2.58	10.98	1.27
T2	± 0.460	± 0.150	± 0.041	± 0.213	± 0.039
	56.13	48.40	2.63	10.55	1.15
T3	± 0.458	± 0.261	± 0.066	± 0.148	± 0.024
3 months	57.35	47.50	2.47	11.23	1.63
T1	± 0.288	± 0.654	± 0.020	± 0.558	± 0.030
	56.45	49.58	2.65	10.70	1.55
T2	± 0.517	± 0.117	± 0.014	± 0.271	± 0.029
	55.50	50.40	2.69	10.25	1.39
T3	± 0.600	± 0.20	± 0.050	± 0.366	± 0.021
4 months	56.10	49.40	2.54	10.62	2.09
T1	± 0.126	± 0.245	± 0.028	± 0.638	± 0.062
	55.90	51.47	2.70	10.25	2.07
T2	± 0.482	± 0.103	± 0.029	± 0.095	± 0.067
	54.72	52.32	2.74	10.03	1.90
T3	± 0.343	± 0.223	± 0.025	± 0.095	± 0.056

T1 = Control
T2 = Sunflower meal (30%)
T3 = Leucaena (30%)

Ripening indices:

Fig (1) referred that T3 cheese showed higher value of SN/TN at the end of storage. While, control cheese as shown in fig (2) showed higher value of total volatile fatty acids.

Fig (3&4) shows the values of FRI and SRI, these values were increased with increasing storage period. However, T3 showed higher value at the end of storage.

Organoleptic properties:

Table (7), shows the sensory evaluation of Domiati cheese during storage. T3 cheese was ranked the highest total score (65/100) when fresh. Also, T3 cheese ranked the highest total score (95/100) at the end of storage period (4 months).

Table (6): Effect of protein source in rations of lactating buffaloes on amino acids composition of Domiati cheese (mg/100 g).

Amino acids	T1		T2		T3	
Essential	**Fresh**	**4 months**	**Fresh**	**4 Months**	**Fresh**	**4 months**
Arg	1.93	Trace	0.62	0.51	7.45	345.4
His	0.75	1.23	3.63	1.57	3.15	0.85
Ile	1.08	3.08	1.17	3.31	2.84	4.76
Leu	Trace	Trace	Trace	5.16	6.41	Trace
Lys	2.35	2.28	9.95	2.74	1.23	59.30
Met	11.46	7.38	2.20	13.80	1.75	0.80
Phe	Trace	100.8	5.76	3.93	8.11	4.08
Thr	0.71	91.38	1.50	Trace	Trace	1.22
Val	0.30	0.71	3.21	26.40	5.84	0.42
Total	**18.58**	**206.86**	**38.04**	**57.42**	**36.78**	**416.81**
Non-essential						
Ala	3.14	14.35	3.89	0.72	Trace	66.51
Asp	0.40	64.54	13.22	20.8	2.28	42.13
Cys	1.48	10.38	9.05	23.94	55.81	11.16
Glu	6.43	1.69	2.90	1.33	4.03	1.33
Gly	1.94	9.49	3.86	4.29	0.97	0.52
Pro	1.53	1.87	7.95	18.86	Trace	399.8
Ser	0.34	0.22	0.24	37.09	1.32	4.23
Tyr	7.48	13.23	9.64	97.5	3.18	12.37
Total	**22.7**	**115.77**	**50.75**	**204.53**	**67.59**	**538.05**

T1 = Control
T2 = Sunflower meal (30%)
T3 = Leucaena (30%)

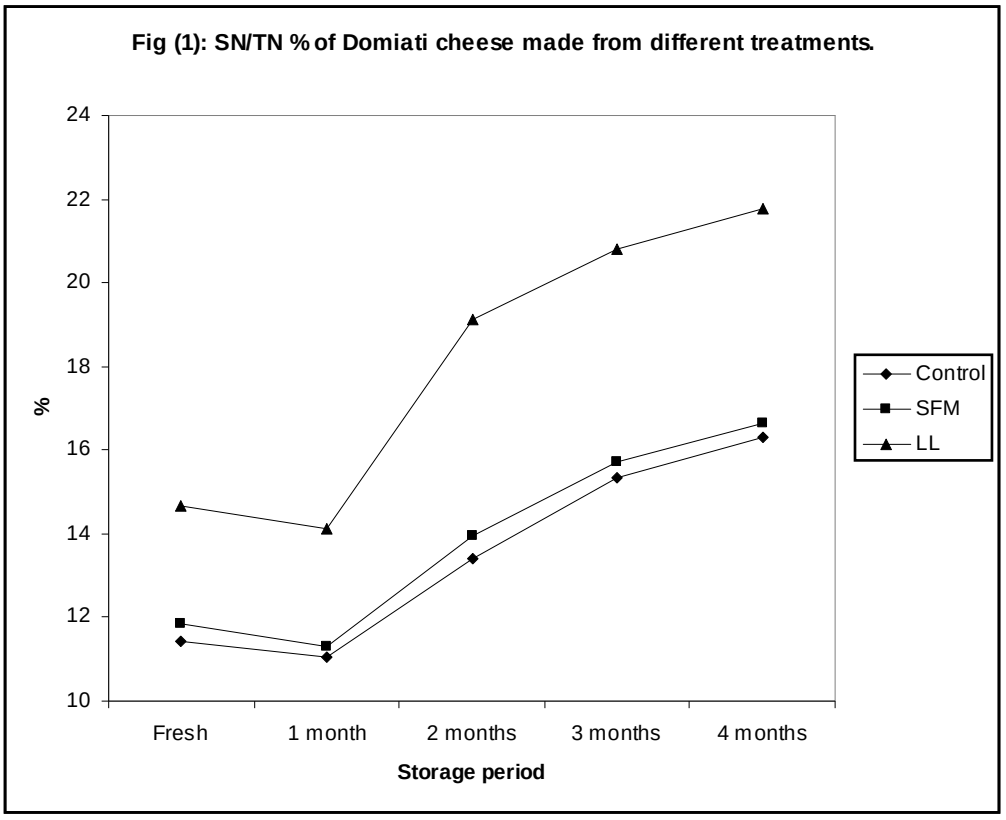
Table (7): Sensory evaluation of Domiati cheese from different treatments (average of three replicates).

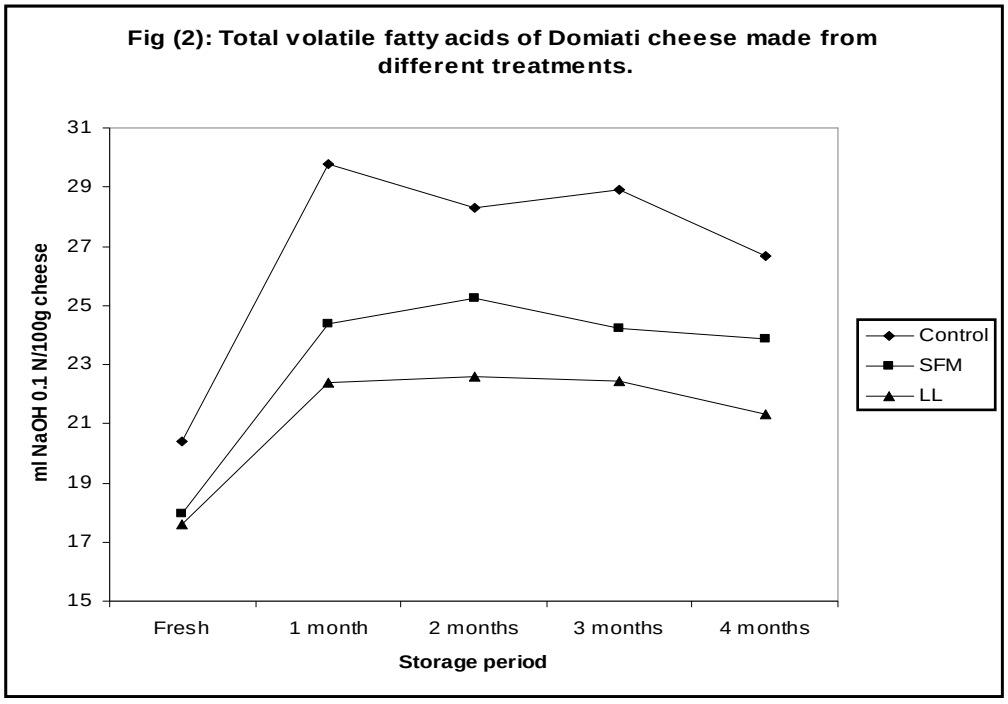
Item	Flavour 60	Appearance 10	Body & texture 30	Total score 100
Fresh				
T1	30	9	19	58
T. 2	35	7	16	58
T. 3	35	10	20	65
1 month				
T1	35	8	18	61
T. 2	35	7	17	59
T. 3	40	10	20	70
2 months				
T1	45	7	21	73
T. 2	48	6	20	74
T. 3	56	8	25	89
3 months				
T1	47	7	22	76
T. 2	50	6	20	76
T. 3	58	8	27	93
4 months				
l T1	51	7	23	81
T. 2	55	6	20	81
T. 3	59	8	28	95

T1 = Control
T2 = Sunflower meal (30%)
T3 = Leucaena (30%)

4. Conclusion:

Using of Sunflower meal and Leucaena leaves as a source of 30% of protein requirements in the feeding system of dairy buffaloes increased total solids, fat, protein and essential amino acids of milk than control one. Leucaena leaves improved the quality of Domiati cheese, so its uses in the feeding system of dairy buffaloes are recommended.





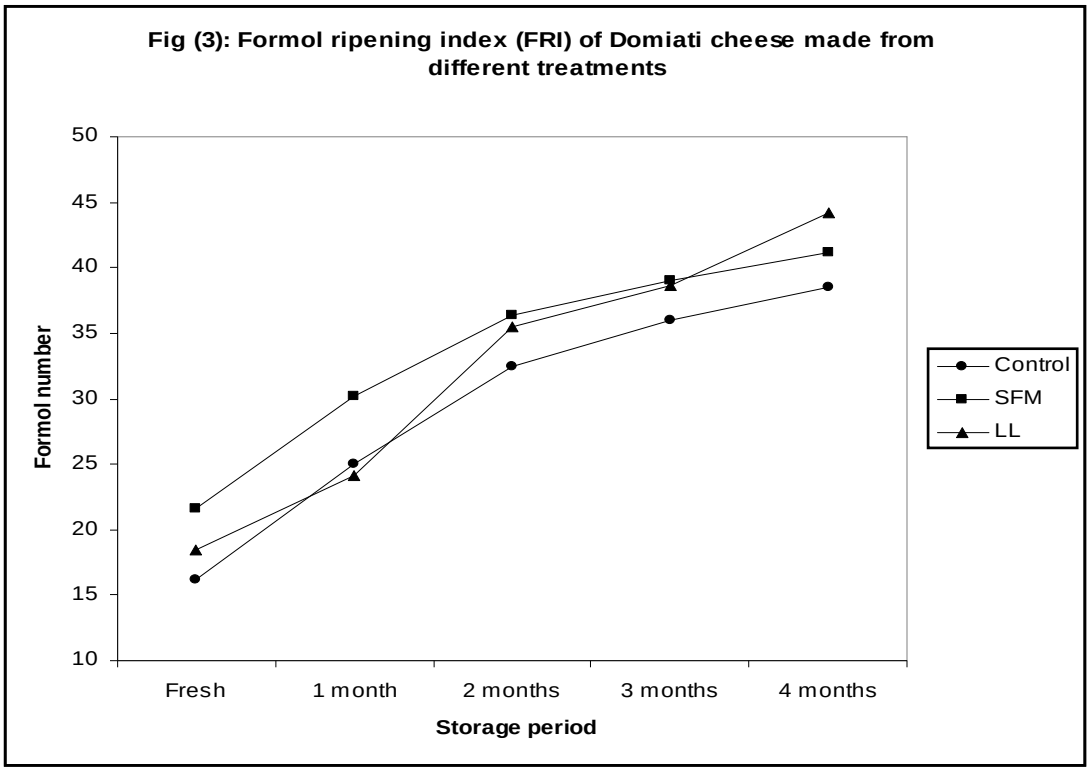


Fig (3): Formol ripening index (FRI) of Domiati cheese made from different treatments
Formol number
50
45
40
35
30
25
20
15
10
Fresh
1 month
2 months
3 months
4 months
Storage period
Control
SFM
LL

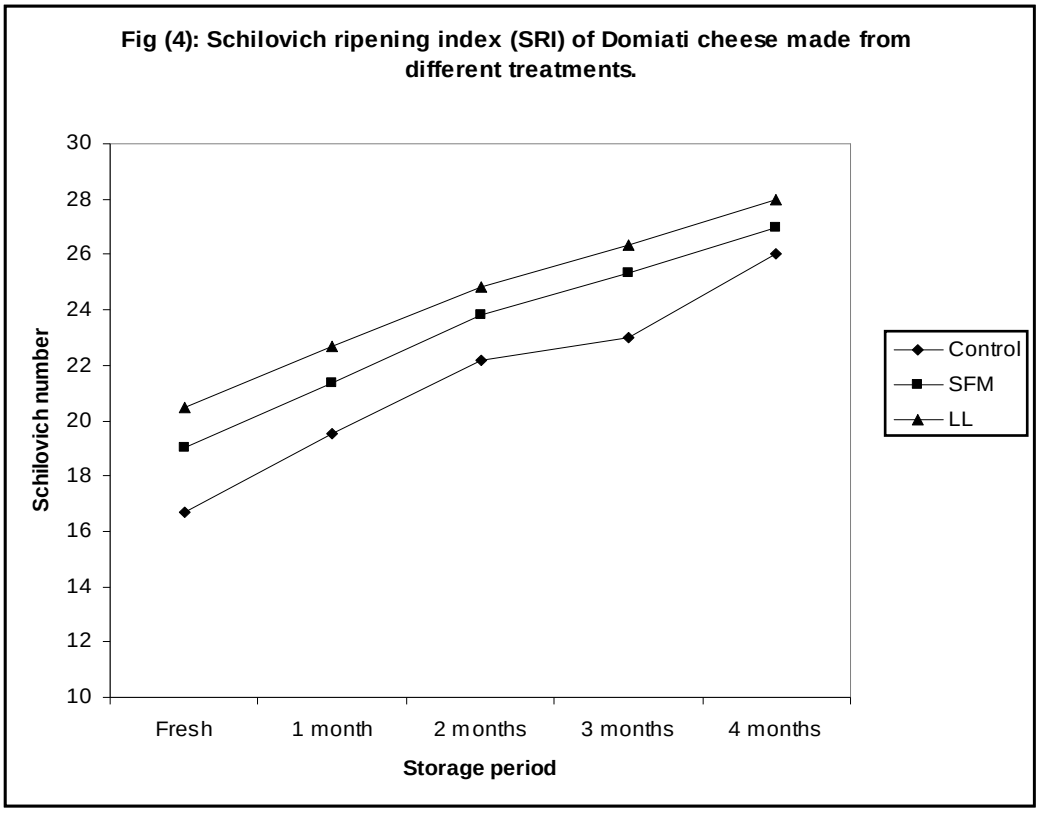


Fig (4): Schilovich ripening index (SRI) of Domiati cheese made from different treatments.
Schilovich number
30
28
26
24
22
20
18
16
14
12
10
Fresh
1 month
2 months
3 months
4 months
Storage period
Control
SFM
LL

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Application of Alpha mapping (α-mapping) of SP well-log Image, to obtain lithology and Correlate to evaluate the Reserves of Shan4 Depression of Shahejie formation China

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Abstract: Deducing geological parameters using SP curves is a very tedious, expensive, and error prone process such as obtaining formation water resistivity and the measurement of small negligible voltage potentials, mud filtrate resistivity and shale volume. This is due to the fact that there are many complex dependent variables surrounding data acquisition using Statistical method of data collection in an SP log image, these variables includes: borehole invasion, shale content, Bed resistivity and the ratio of salt water mud(R_{mf}) and fresh water mud (R_w). We have used Alpha (**α**) mapping method of SP-Log considering the shale content of the formation and the maximum possible deflection of Sp that a thick shale free porous and permeable formation can have at a given ratio of R_{mf} and R_w to obtain the lithology of Shan4 depression as well as limit error to the bearest minimum at a low cost of acquiring the petrophysical parameters. Based on the Structure map Shan4 depression in shahejie formation is composed of a complex depositional system of a prograded elongated delta, beach and bar formed under lower current energy of a shore-shallow lake. Hydrocarbon trap is created by an anticline pool separated by numerous oil layered complex faults, Oil and seeps in the depression are found in Tertiary sandstone reservoirs as well as underlying basement located at an approximate depth of 2020m below sea level (-2020m), including the Jurassic sandstone reservoirs and the carboniferous –Permian and Ordovician weathered zone.
[Taiwo Olusoji Lawrence. **Application of Alpha mapping (α-mapping) of SP well-log Image, to obtain lithology and Correlate to evaluate the Reserves of Shan4 Depression of Shahejie formation China**. Journal of American Science 2011;7(1):291-299]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: SP well-log; Shahejie formation; lithology; correlate

1. Introduction

An oil or gas prospect is developed by extensive and very expensive studies of regional geology, including subsurface geology and geophysics. Tile exploratory wells may cost several millions dollars to drill. It would be foolish to risk the loss of a prospect as a result of ignorance or carelessness on the part of the driller, the engineer, or the well-site geologist. Spontaneous potential (SP) is one of the earliest logs used and still in use the first was run in 1927 Spontaneous log records the electric potential set up between two electrodes in a sonde drawn up the borehole and fixed electrode at the earth's surface (Richard 1998), it is mostly used for the determination of lithology of a formation, to obtain a region that can be categorized as a reservoir and non reservoir. It is also mostly used in correlation, this article has grossly explored this advantage, the raw data (logs Image) are combined in a detail interpretation to obtain values of geological parameters.

The first commercial oil discovery was made in early 1960s. Since 1984 (S.Zhang et al 2004). Shan4 depression in shahejie formation is composed of a complex depositional system of a prograded elongated delta, beach and bar formed under lower current energy of a shore-shallow lake. Hydrocarbon trap is created by an anticline pool separated by numerous oil layered complex faults. No.1 fault. Formation water geochemistry and overpressure transference also signify that the No. 1 fault was the main hydrocarbon migration pathway. Besides the long-term growth fault, early and late active faults also affect hydrocarbon migration and accumulation. The formation of the Shahejie oil pool is characterized as an autochthonous, hydrocarbon migration model controlled by overpressures, (C. Zhang et al., 2006). Source rocks of Shan4 are deep-water lacustrine shale of Eocene and Oligocene age that are draped over the buried hills. The secondary member is made up of sandstone, lithology for reservoir is silt-fine sandstone. It is up to 25%~30% for the porosity, and it is generally less than 120 mD for permeability of sandstone. It is about 10-15% for shale content. The thickness of the secondary member of the Shahejie Formation is approximately 1,000m in each sub-basin and its total organic carbon (TOC) values range from about 1 to 4.5. Oil-source rock correlations clearly demonstrate that oil in the buried hills was derived from adjoining lacustrine shale and mudstone (USGS 2000), its contact of oil and water is approximately located on 2160m in depth. The best reservoir rocks are shallow marine dolomite and limestone beds of Proterozoic, Cambrian, and Ordovician age that have been exposed to long periods of sub aerial weathering, erosion, and karst processes. Solution-enlarged fractures, vugs, and cavities are common features in

the better reservoirs. These dolomite and limestone reservoirs occupy the Proterozoic Wumishan Formation and numerous formations of Cambrian and Ordovician age. Local reservoirs consist of Archean crystalline basement, Proterozoic/lower Paleozoic sandstone beds, and Mesozoic volcanic rock and sandstone. The lower members consist of red mudstone interbeded with thin sandstones. There exist a localized unconformity at the top of the lower part of the member above which there is a bluish-grey mudstones siltone and sandstone with gypsum and halite horizons, near the top of the member there are also carbonates and oil shales. This member is typically 350-950m thick with the thickness in the northern part of Dongying Sag. (M.B. Allen et al., 1997).

Existing geophysics exploration methods (magnetic, gravity, seismic and electromagnetic) are based on the geophysical properties of the subsurface. However, these methods are limited in scope since each one of them is only applicable to one of these properties at a time. For example the seismic method is related to the thickness and density of a substance since it deals with the velocity of waves in a given material (S. Morris Cooper et al 2010), while well-log is all encompassing, it depends on the lithology of the formation, this paper hopes to describe the reservoir formed in shahejie formation by using hand correlation of the well-log on Corel draw software to obtain the data in the various wells as well as using a simple software surfer to create the grid based maps. We shall use the surfer software to create the sandstone thickness map, structure map, and the Oil saturation Map of the formation, the aim of this paper is to create a simple model of the overall architecture of the formation which will help predictions of the structure, stratigraphy and hydrocarbon potential of the formation.

2. Study Area

Shan4 depression is located within the giant Shengli petroleum province in east China, the source rock in the basin is mainly the lower part of the third member (E_S3) of the Eocene portion of the Shahejie Formation with other sags (smaller sub-basin) around it. Most basins in this formation lie in the Eocene portion except Bozhong sub-basin as narrated by F. Hao et al., (2009). The Shahejie Formation is subdivided into four members；they are the first member (E_S1)，the second member E_S2，the third member Es3，and the fourth member Es4 from the upper to the lower．Delta，beach bar, and braided fan sediments develop in E_S4 member．The provenance comes from the northern，southwestern，and southern uplifts．The lithologic associations are mainly siltstone，fine sandstone and medium sandstone (Zhang 2008). Tertiary anticline demarcated by faults formed the major hydrocarbon source and reservoir rocks, more reservoir rocks occur in the footwall crest of tertiary faults blocks. Existing descriptions of Shahejie formation tend to account for its structure in one of two ways: (1) orthogonal extension, creating NNE trending normal faults as the result of ESE-WSW extension (Hu Jianyi et al.,1989); (2) as a dextral pull-apart structure created at the fault-overstep of Tianhang Shan and Tan-Lu(Tan-cheng-Lujiang) faults systems at the western and eastern margins of the formation. Shahejie formation was deposited across a wide area of the Bohai Basin and for the first time in the Cenozoic, significant thickness of sediment accumulated in the Bozhong Depression and the northern part of the Huanghua, the formation reaches thickness over 3000m . (M.B. Allen et al., 1997). The main minerals are quartz, feldspar, and cuttings. Among these, quartz accounts for 25%–35%, averagely 26%; cutting 30%–45%, averagely 39%; feldspar 20%–45%, averagely 35%. Fillings are mainly calcite and clay. Generally, carbonate cement content is below 10%, locally up to 30%; and clay content is mostly between 3%–8%, highest up to 25% (*ZHANG Wen-cai et al., 2008*). The well location (geodetic coordinates) of 22 wells has been considered for this paper (figure 1).

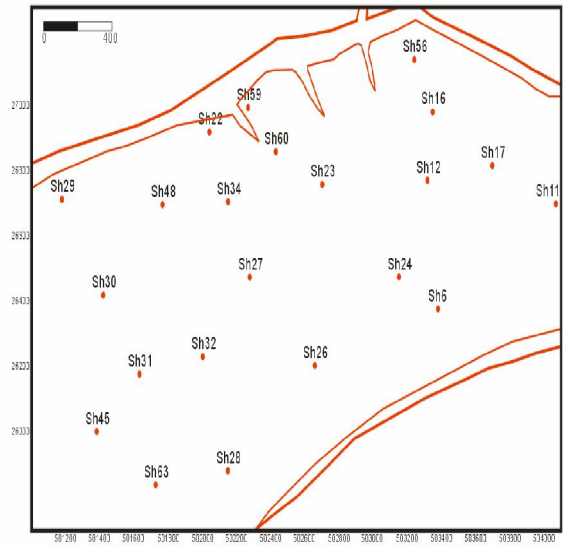


Figure 1: Base map of Study Area

3.0 Lithology of the formation interpreted by α-mapping of SP Well-logs

A well log is a record of formation characteristics made by a tool as it rises in a wellbore. They provide a means to evaluate certain formation parameters and the hydrocarbon production potential

of a reservoir. The principal geological application of logs has been known to be used for the understanding and description of the subsurface stratigraphy and correlation. The SP log is a measurement of the natural potential differences or self potentials between an electrode in the borehole and a reference electrode at the surface. The response of the SP curve in front of fractured zones may exhibit either erratic behavior or a more systematic negative deflection due to a streaming potential (the flow of mud filtrate ions into the formation), however, streaming potentials can also occur near silt beds (Crary et al., 1987).

The method presented here takes advantage of shale content in the formation and the static Spontaneous Potential (SSP) curve, the maximum possible deflection of Sp that a thick shale free porous and permeable formation can have at a given ratio of Rmf and Rw. Here Alpha (**α**) mapping method of SP-Log was employed to limits the error to the lowest minimum.

Both the SP and Gamma Ray log can be used to map "clean" sediments, which generally have higher permeability and porosity, the SP response is depressed by shale, thin beds and hydrocarbons. SP alpha mapping is be used to determine clean zones (shale free), assuming a lower response is produced only by shale.

The first step is to compute the Static SP (SSP), which is the ideal SP response for clean, thick rocks, using the expression below:

$$SSP = (0.133 * T_f + 60)\log\left(R_{mf} / R_w\right)$$

Then we Pick the shale cutoff (**α=0**%) of the SSP (can drift over long distances, of little consequences for single formations) and draw a line on the log then obtain two alpha values at , **α**=75% and **α** =50% taken with relative to the shale base line (as shown in Figure 2 above), permeable zones are indicated where there is a deflection from the shale base line (this don't necessarily means that where there is no deflection are impermeable) (USGS 2000).

4.0 Well log correlation Using Corel Draw

Well correlation is used to establish and visualize the lateral Extent and the variation of reservoir parameters. In carrying out a correlation we subdivide the objective sequence into lithologic units and follow those units or their generic equivalent laterally through the area of interest. The scarcity of digital data, at a point of neccessity poses a major threat to the application of computer methods for regional or field wide correlation. Previous methods of automated correlation could handle very simple relative vertical displacement but often had limited success in accommodating missing sections and differential stretch of correlative intervals between wells, these features results from a normal geological history that includes multiple regime of erosion and non-deposition, which hamper periods of continuous varying rates of sedimentation (John et al.,1992). This papers hopes to take into cognizance the existence of these gaps and stretch problems, and thereby solving it by Alpha mapping of SP log by hand correlation. Though manual correlation is labor intensive, it gives a hope to the poor countries like Africa where geologist lack the funds to obtain the very expensive software program such as Petrel by Schlumberger, this make it very difficult for this geologist to study the petrophysics of the subsurface of their wildcat. This paper hopes to present the use of very common software in our desktop. Corel draw is graphic software we utilize for the purpose of correlation considering the lithology obtained from the α-mapping of SP well-logs.

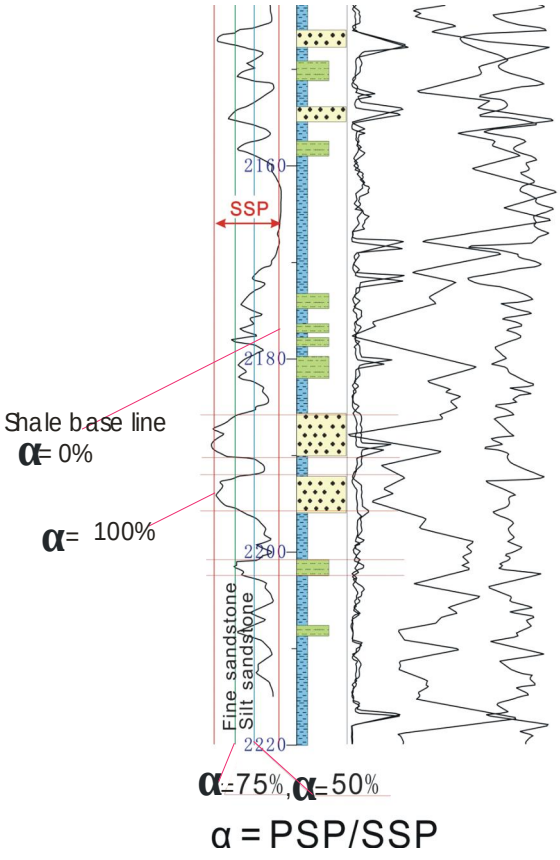


Figure2: Interpretation Techniques of α-mapping of SP Well-logs

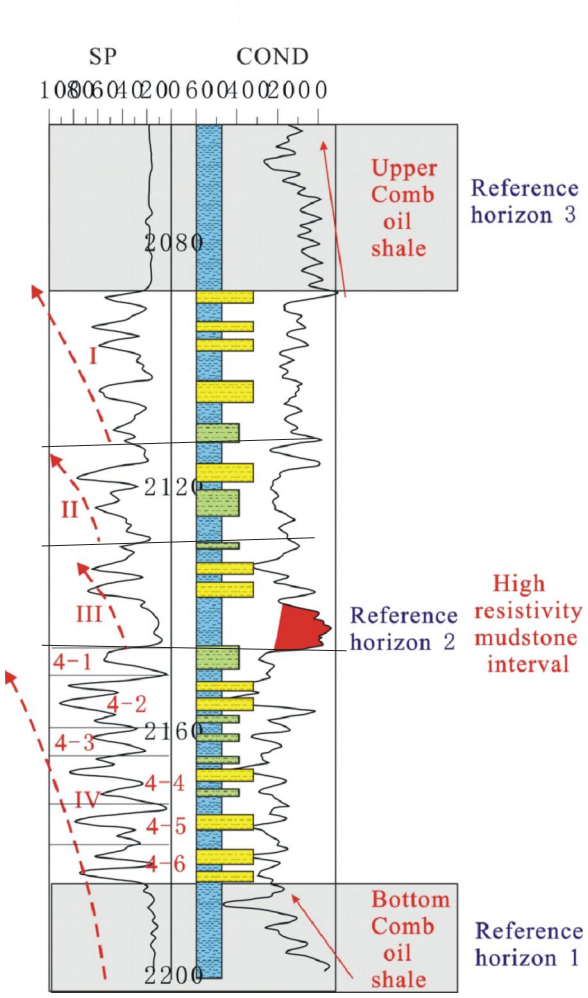


Figure 3: Well-log Profile showing division of the fouth package into six para-sequence

Correlation was done from near to far in the light of net sections, To start the correlation process we take the set of logs and select a datum plane (by comparing the log response. This is a marker which can be traced through all data points. A good datum plane would be a continuous shale because we assumed that it represents a "flooding surface" present over a wide area. Since shales are low energy deposits we may also assume that they have been deposited mostly horizontally, blanketing the underlying sediments thus "creating" a true datum plane, to align all logs at the datum plane this becomes a straight horizontal line. Note that by doing so we ignore all structural movements to which the sequence has been exposed (Figure 4). Six sections were chosen to form cross section net, the marks and cycle was determined. Six cycles with the scale of Para-sequence set and three marks was determined in the area. The six cycles constitute six sand packages (from up to down it is the I/II/III/IV /V/VI for the code of four sand packages (figure 3). This is standard well for well of Sh29, Sh28, Sh34 and Sh12, Sh24, Sh11 the zonation has been done, it is important to divide packages 3 and 4 into six cycles with the scale of the para-sequence, though this paper will be limited to the fourth package and the first Para-sequence (figure 4), it is recommended that more research should be done on the other packages.

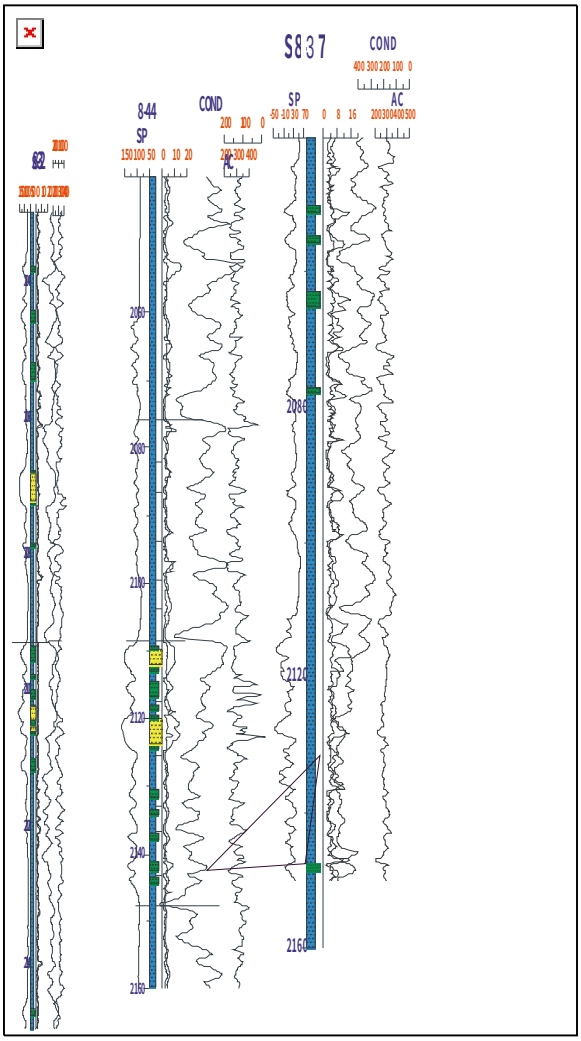


Figure 4: Correlated section

5.0 Parameter interpreted by logging

In the old days the calculation of R_w and S_w, Φ were made using company chart book and a calculator. Today, however, computer-processed interpretations, which give continuous readings of lithology, porosity and the percentages of various fluids. Although computer output looks very convincing the idea of baggage in baggage out should not be taken for granted. Here we take into consideration few petrophysical parameters, with

logs together with conventional logs it is possible to calculate essential reservoir parameters as well as identify and understand diagenetic phenomena within reservoirs, as researcher's belief that formation evaluation is now entering its third age, (Richard 1998). Reservoir management strategies are as realistic as the "image" of spatial distribution of rock properties. Permeability is the most difficult property to determine and predict, many investigators have attempted to capture the complexity of permeability function in models with general applicability. Empirical models are based on the correlation between permeability, porosity, and irreducible water saturation (Shahab 1997). The Parameters interpreted by logging includes:

- The top elevation of the fourth package and the first Para-sequence sandstone (datum elevation) = (Kelly bushing + surface elevation) – the depth of 4-1 sandstone.
- Sandstone thickness = bottom depth – top depth the fourth package and the first Para-sequence sandstone.
- Porosity, permeability, oil saturation determine by (Archie equation) and electric facies of the fourth package and the first Para-sequence sandstone was calculated by logging data which was read out from well profile.

Oil saturation was computed using the popular Archie equation of water saturation (Sw), then Oil saturation (So) = 1-Sw

$$S_w = \left(\frac{a * b * R_w}{\Phi^m * R_T} \right)^{\frac{1}{n}}$$

Where

a - tortuosity factor, a =1;
b - lithologic factor, b = 1.
m - cementation exponent, m = 2;
n - saturation exponent, n =2;
Rw - resistivity of formation water =1.98
Porosity was obtained from the expression:

$$\Phi = 13.978 + 0.02632\,AC$$

Where AC is the Acoustic log reading for a particular well
Permeability was obtained by the expression:

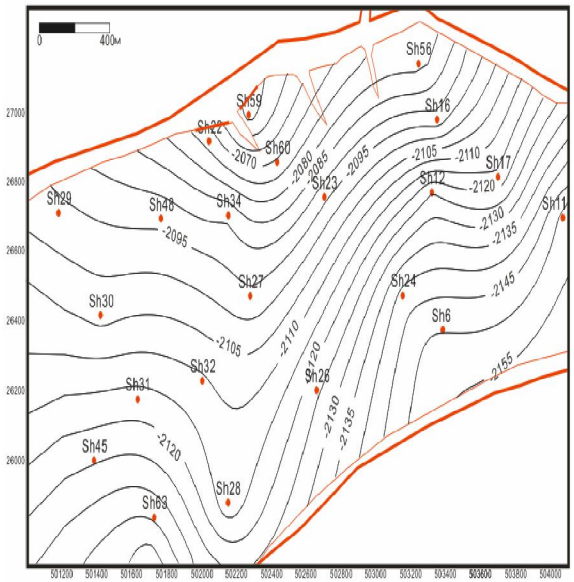
$$InK = 4.174 - 0.44\,In\Phi + 0.125\Phi$$

(USGS 2000).

In general, reservoirs in the deep horizons in Shan4 depression consist of medium-porosity Medium-permeability sandstones. The porosity is generally between 3%–22%, and the permeability is mostly between 0.1×10^{-3}–250×10^{-3} μ m2. Nevertheless, in some intervals in the deep horizon sandstone, there are well developed reservoirs. For example, the porosity of well Sh16 in the interval from 2115 m to 2130 m is an average of 21%, and the highest permeability is up to 250×10^{-3} μ m^2.

6.0 Contour map

Surfer is particularly utilized to create structure maps, Sufer is a software used to create a grid based map, the Krigging griding method was considered, Kriging is a geostatistical gridding method that has proven useful and popular in many fields. This method produces visually appealing maps from irregularly spaced data. Kriging is a very flexible gridding method can be custom-fit to a data set by specifying the appropriate variogram model. Within Surfer, Kriging can be either an exact or a smoothing interpolator depending on the user-specified parameters. It incorporates anisotropy and underlying trends in an efficient and natural manner, and underlying trends in an efficient and natural manner.



Fault

Figure 5: Structure Map

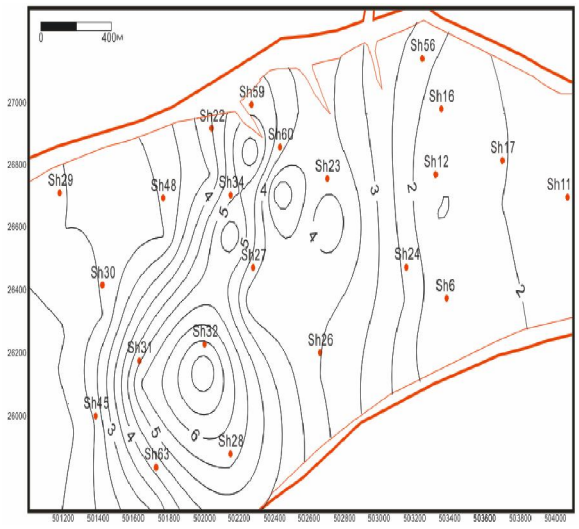


Figure 6: Sandstone Thickness Map

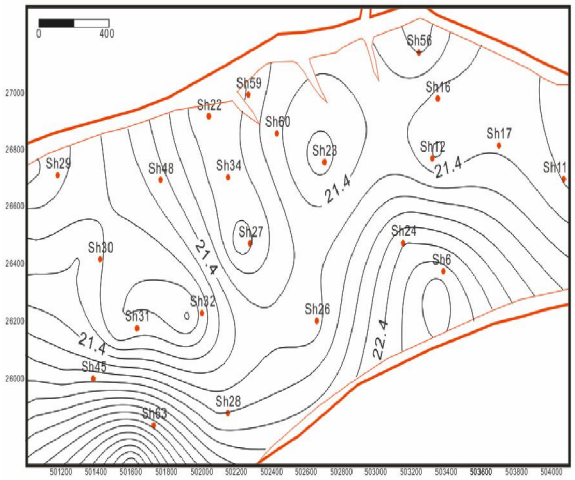


Figure 7: Porosity Map

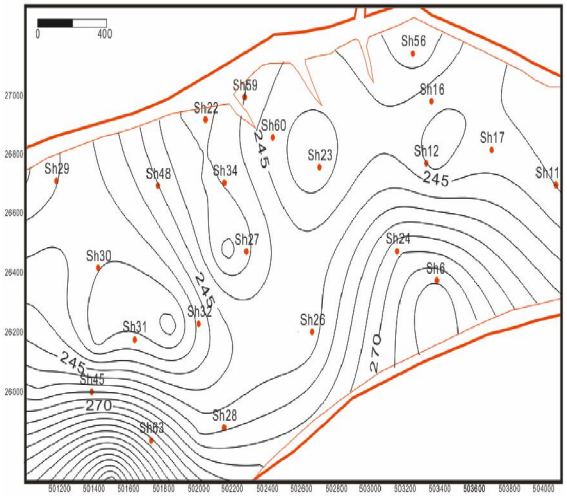


Figure 8: Permeability Map

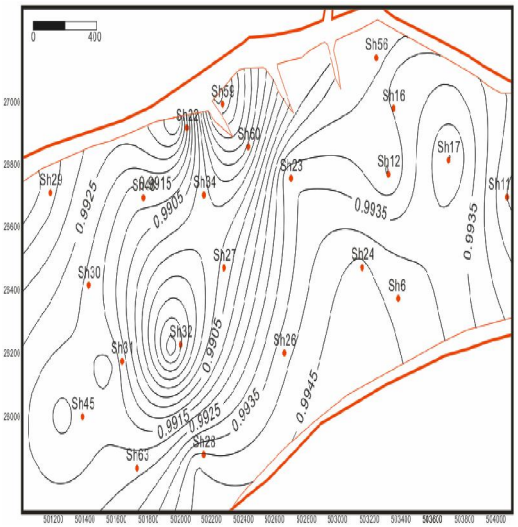


Figure 9: Oil saturation Map

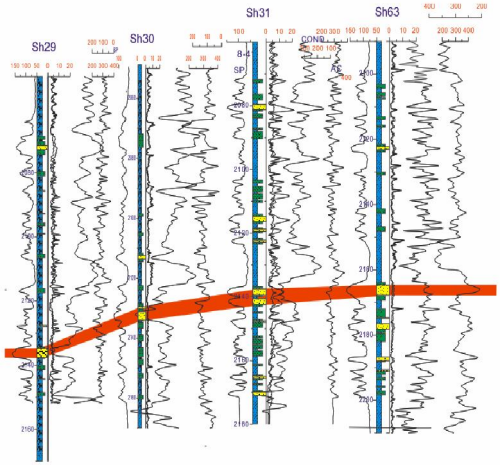


Figure 10: Cross Section of the pool in well Sh29, Sh30, Sh31 and Sh63.

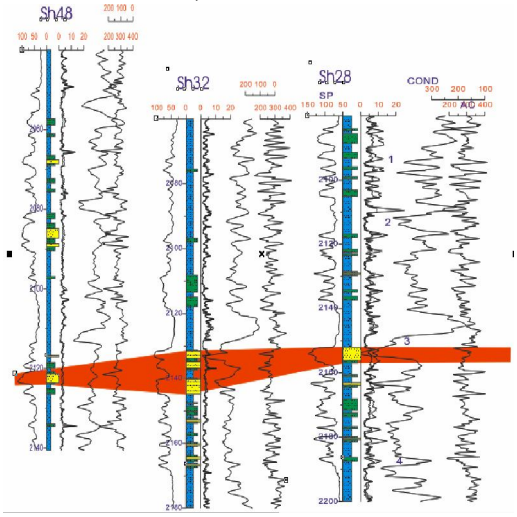


Figure 11: Cross Section of the pool in well Sh48, Sh32 and Sh28.

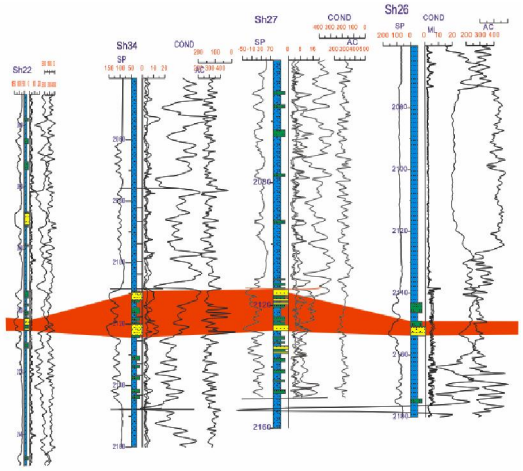


Figure 12: Cross Section of the pool in well Sh22, Sh34, Sh27 and Sh26.

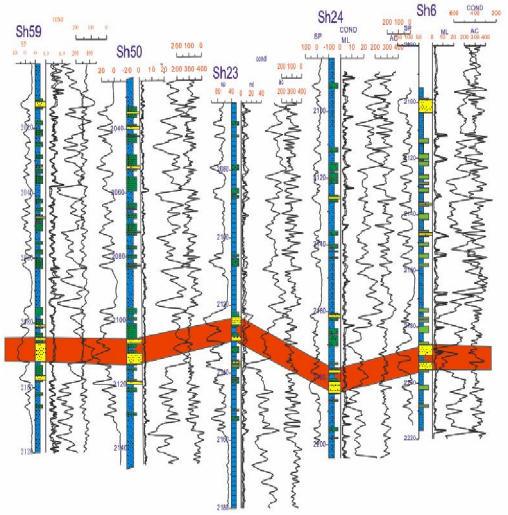


Figure 13: Cross Section of the pool in well Sh59, Sh50, Sh23, Sh24 and Sh6.

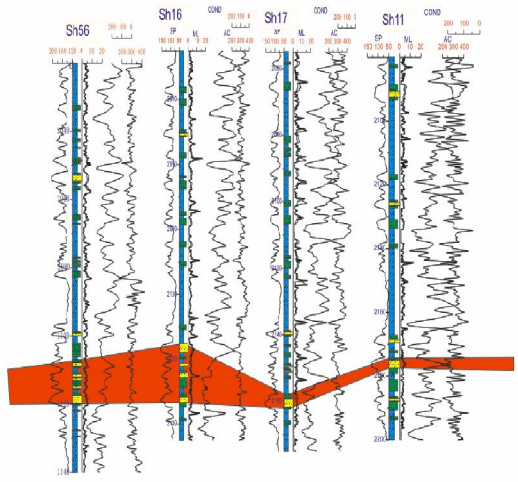


Figure 14: Cross Section of the pool in well Sh66, Sh26, Sh23, Sh24 and Sh6.

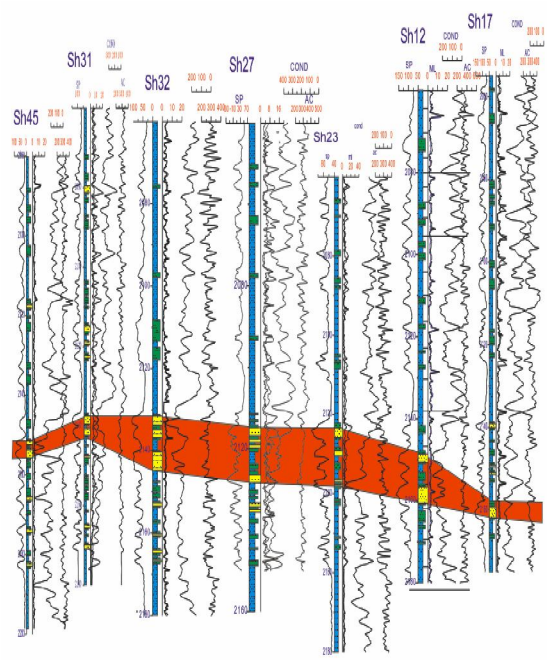


Figure 15: Cross Section of the pool in well Sh45, Sh316, Sh32, Sh27, Sh23, Sh12 and Sh17.

7.0 Conclusion

Nearly all anticlinal reservoirs are broken by faults. Usually they are at an angle of 70 degrees to the axis of the anticline, they are normally seals and break the reservoirs into individual units, each with its own pressure and oil-water contact. Obviously for efficient production each fault block must be considered a separate reservoir. Oil and seeps in the depression are found in Tertiary sandstone reservoirs as well as underlying basement, including the Jurassic sandstone reservoirs and the carboniferous – Permian and Ordovician weathered zone. Oil are produced from several faulted blocks shown in the structure map (figure 5), this result agrees with previous work in Shahejie formation particularly Fulin Basin by (Chen Jianyu et al., 1996). Based on the Structure map Shan4 depression in shahejie formation is composed of a complex depositional system of a prograded elongated delta, beach and bar formed under lower current energy of a shore-shallow lake. Wells for Tertiary reservoir failed because of inaccurate prediction of the oil-bearing height in the Jiyang Depression. (Zhao 2010). Hydrcarbon trap is created by an anticline pool separated by numerous oil layered complex faults, Oil and seeps in the depression are found in Tertiary sandstone reservoirs as well as underlying basement located at an approximate depth of 2020m, including the Jurassic sandstone reservoirs and the

carboniferous –Permian and Ordovician weathered zone. The oil-bearing height actually reflects the amount of hydrocarbon charging. While the charging amount of hydrocarbon mainly depends on the charging force of the hydrocarbon to the trap (Zhao et al., 2010). It is composed of organic-rich dark mudstone and oil shale of lacustrine origin and contains good quality Type I-II kerogen, all the oil in this member originated from Es3 source rock as extrapolated from biomarker similarity functions between oils and the source rock extracts. Assessment of the oil generation history indicates that oil generated is expelled from South slope of the Bohai Bay Basin east China. Though Xiongqi et al (2005) suggested that this suggest significant hydrocarbon expulsion from mature sourece rocks in the fourth (Es4) and third members (Es3) of the Oligocene-Eocene Shahejie Foemation in the Niuzhuang Sag.

Shan4 faulted zone which could trap high quantity of oil pool are: Sh56, Sh50, Sh6, Sh16, Sh34, Sh27, Sh32, Sh27, Sh23 and Sh12 as the distance between the trap and the source rock is not too far. When the distance between source rock and trap and the altitude difference between source rock and trap increases, the actual migration distance of hydrocarbon will become longer, the dispersed rate of the source rock will increase, and the amount that can enter into the trap will decrease (*Zhao Leqiang et al., 2010*). A little amount of oil can also be gotten from well Sh59, Sh23, and Sh24. The high porosity distribution of Shan4 (figure7) indicate the wide variety of grain distribution, interstitial clays and secondary silica, size and shape of the pore. The analytical data (table 1) showing the porosity and permeability distribution shows that they are fairly good source rock. This results agrees with findings in other depressions in Shahejie formation Xiongqi et al., (2005) he discovered that most of the hydrocarbon are generated and expelled from Shahejie formation source rock in Niuzhuang Sag, and are made up of algae-rich Saline-brackish water lacustrine, Es4 oil Shales of the Shahejie forrmation.he also noted that all the oils in the formation share some common characteristics of brackish to saline lacustrine sourced oils, featuring high sterane and ammacerane contents, high C35/C34 homohopane ratios and low diasterane contents. Xiongqi et al (2003).

The findings agrees with (Richard 1998), (J. Zhang et al., 2008) that logs can also be used to determine the facies and depositional environment of a reservoir, the geometry and orientation of reservoir can be predicted.

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Simple Novel Spectrophotometric and Spectrofluorimetric Methods for Determination of Some Anti-hypertensive Drugs

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Abstract: Accurate, precise and selective spectrophotometric and spectrofluorimetric methods were developed and subsequently validated for determination of Torasemide (I), Irbesartan (II) and Olmesartan medoxomil (III), where (I) could be determined in presence of its acidic-degradate as stability indicating method, utilizing derivative ratio spectrophotometry, also in human plasma it could be determined by spectrofluorimetric method, (II) could be determined in a binary mixture with Hydrochlorothiazide (HCTZ) by simultaneous determination, utilizing ratio subtraction and spectrofluorimetric techniques, while (III) could be determined in presence of its alkaline-degradate as stability indicating method, utilizing derivative ratio and pH-induced difference spectrophotometric technique, also in a binary mixture with Hydrochlorothiazide (HCTZ), it could be determined by simultaneous determination, using ratio subtraction and spectrofluorimetric methods. All the proposed novel methods were validated according to International Conference of Harmonization (ICH) guide lines and successfully applied to determine the mentioned studied drugs in pure form, in laboratory prepared mixtures and in pharmaceutical preparations. The obtained results were statistically compared to the reference methods of analysis [for I, II and III, respectively] and no significant difference were found.

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Keywords: Torasemide, Irbesartan, Olmesartan medoxomil, Derivative Ratio, Ratio subtraction, Difference Spectrophotometry, Spectrofluorimetry, Stability Indicating and Simultaneous Determination Methods.

1. Introduction:

Torasemide (I) is (1-isopropyl-3-[[4-(3-methylphenylamine) pyridine]-3-sulfonyl] urea) a loop diuretic, mainly used at low doses for the management of hypertension, where in large doses used for management of oedema associated with congestive heart failure[(1)]. Irbesartan (II) is 2-butyl-3-[[2-(tetrazol-5-yl) biphenyl-4-yl]-methyl]-1,3-diazaspiro[4.4]non-1-en-4-one, acts as an

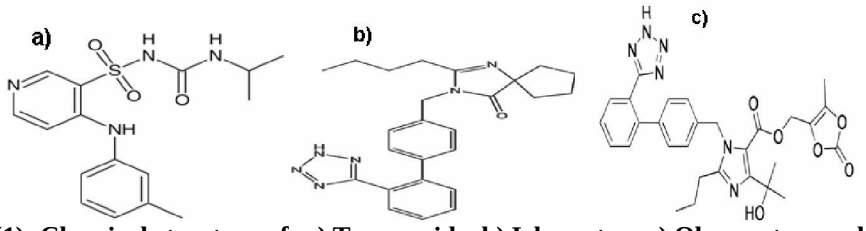

Figure (1): Chemical structure of: a) Torasemide, b) Irbesartan, c) Olmesartan medoxomil

angiotensin-II receptor antagonist, used mainly for the treatment of hypertension[(2)], while, Olmesartan medoxomil (III) is 5-methyl-2-oxo-1,3-dioxolen-4yl) methyl-4-(1-hydrxy-1-methylethyl)-2-propyl-1-[4-(2-(tetrazole-5yl)phenyl] methylimidazole 5 carboxylate, used for the treatment of hypertension by the same mechanism as (II)[(3)]. The ICH-guide lines[(4)] recommends performing stress-testing of the drug substance that can help in identifying the likely degradation-products, also can be useful in establishing the degradation-pathways and validating the stability-indicating power of the analytical procedures used[(5)]. Stability-indicating methods can be used for evaluating the drug in the presence of its-degradation products, excipients and additives [(6)]. Several methods have been reported for the determination of (I), including

colorimetry[7], differential-pulse adsorptive stripping voltammetry[8], capillary zone electrophoresis (CZE)[9,10], gas chromatography[11], micellar liquid chromatography[12], and high-performance liquid chromatography[13-22]. Alone or in combination with HCTZ, Irbesartan has been determined by derivative spectrophotometry[23-27], kinetic Spectrophotometry[28], spectrofluorimetry[29], colorimetry[30], adsorptive stripping voltammetric[31], A differential pulse (DP) and square wave (SW) voltammetry[32], capillary zone electrophoresis[33-35], micellar-electrokinetic chromatography[36], and high-performance liquid chromatography[37-43]. While for Olmesartan medoxomil (III), several methods have been reported for its determination, either alone or in combination with HCTZ, these methods were based on absorption ratio spectrophotometry[44], ratio spectra derivative and zero-crossing difference spectrophotometry[45,46], derivative spectrophotometry[47], direct spectrophotometry[48,49], capillary zone electrophoresis[50], high performance thin layer chromatographic method[51,52], and high-performance liquid chromatography[52-59].

The main goal of this work is to establish accurate, precise, rapid and reproducible spectrophotometric and spectrofluorimetric methods for determination of (I) and (III) in presence of their-degradates, also simultaneous determination of (II) and (III) separately in binary mixture with HCTZ, which can be adopted for the routine quality control analysis of the investigated drugs in raw material, and pharmaceutical preparations as well as for stability studies.

In this paper, between the adopted new spectrophotometric methods, we utilized a ratio subtraction spectrophotometric technique for simultaneous determination of two binary mixtures [(II) and (III)] each with HCTZ.

This technique has the following theory:

A mixture of two drugs X and Y with overlapping spectra can be resolved by ratio subtraction, if the spectrum of one drug, say (Y) is extended more than the other, say (X) can be determined by dividing the spectrum of the mixture by a certain concentration of Y as a divisor (Y'). The division will give a new curve that is represented by:

$$X / Y' + \text{Constant}$$

If the constant is subtracted, then the new curve obtained is multiplied by Y', the original curve of X is obtained.

This can be summarized in the following equations:

$$(X+Y) / Y' = (X / Y') + (Y / Y') = X / Y' + \text{Constant}$$

$$X / Y' + \text{Constant} - \text{Constant} = X / Y'$$

$$X / Y' \times Y' = X$$

The constant can be determined directly from the curve (X+Y) / Y' by the straight line that is parallel to the wavelength axis in the region where Y is extended.

2. Materials and methods

2.1. Chemicals and reagents

Torasemide was kindly provided by Apex Pharma-Egypt and certified to contain 99.70%. Examide® tablets: batch number: MT1120410, manufactured by Apex Pharma-Egypt Company. Each tablet was labeled to contain 20 mg of Torasemide. Irbesartan was kindly obtained by Sanofi-Aventis Egypt and certified to contain 99.90%. Co-Approval® tablets: batch number: 1145, manufactured by Sanofi-Aventis Egypt. Each tablet was labeled to contain 300 mg of Irbesartan and 12.5 mg Hydrochlorothiazide. Hydrochlorothiazide (HCTZ) was kindly provided by Multi-Pharma Egypt and certified to contain 99.50%. Olmesartan medoxomil was kindly provided by Apex Pharma-Egypt and certified to contain 99.70%. Erastapex® tablets: batch number: MT3241009, manufactured by Apex Pharma-Egypt Company. Each tablet was labeled to contain 40 mg of Olmesartan medoxomil. Erastapex Plus® batch number MT0280110, manufactured by Apex Pharma-Egypt Company. Each tablet was labeled to contain 40 mg Olmesartan and 12.5 mg HCTZ.

Boric acid (Adwic), Bi-distilled water, Chloroform, Ethyl acetate and Methanol (Riedel-dehaen, Sigma-Aldrich, Germany), Hydrochloric acid (BDR), aqueous 0.1M, Sodium hydroxide (BDR), aqueous '0.1M and 6.6M; O-phosphoric acid, Potassium Chloride, Potassium Monobasic Phosphate (Adwic) and Sulfuric acid (BDR), aqueous 5.0 M.

All chemical and reagents used through this work are of spectroscopic and spectrofluorimetric analytical grade. Bi-distilled water is used throughout the whole work and is indicated by the word "water".

2.2. Instruments

A double-beam Jasco (Japan) UV/Visible spectrophotometer model J-760, connected to ACER compatible computer and a LaserJet printer is used. The bundled software is spectra manager Jasco (J-760) Version-2. The spectral bandwidth is 0.2 nm and the wavelength scanning speed was 1000.0 $nm.min^{-1}$. The absorption spectra of the reference and the test solutions are recorded in 2.0-mL quartz cells at 25.0 ^0C, using ‘$\Delta\lambda$ = 4 nm and scaling factor of 10 for computing first derivative (D^1).

A spectrofluorimeter (BIO-TEK Kontron, Switzerland) Model SFM25 connected to IBM compatible PC. The Bundled software was WIND25 personal spectroscopy software. The excitation and

emission spectra were recorded over the range of 200 – 800 nm at room temperature.

A (Jenway 3510, UK) pH-meter, equipped with combined glass electrode for pH adjustment.

2. 3. Standard Solutions

2.3.1. Standard solutions of the studied drugs

For spectrophotometric technique, stock standard solutions of (I), (II) and (III), each having concentration of (0.5 $mg.ml^{-1}$) were prepared respectively in 0.1M HCl, methanol and phosphate buffer pH 7, used as working standard solutions. While, stock standard solutions of (I), (II) and (III), each having concentration of (0.1 $mg.ml^{-1}$) were prepared respectively in methanol, used as working standard solutions for spectrofluorimetric technique.

2.3.2. Standard solution of Hydrochlorothiazide:

Stock standard solution of HCTZ having concentration of 0.5 $mg.ml^{-1}$ was prepared in methanol, and used as a working standard solution.

2.3.3. Standard solution of degradates

2.3.3.1. Standard solution of Trosemide acid-degradate

Standard solution of (I) acid-degradates was prepared by mixing 50 mg of authentic (I) with 10 ml 5M sulfuric acid, refluxing for 12.0 hours, cooling, neutralizing with the media with 6.6M sodium hydroxide, and increasing the volume to 100 ml with 0.1M HCl to obtain a concentration of 0.5 $mg.ml^{-1}$.

2.3.3.2. Standard solution of olmesartan alkaline-degradate

Standard solution of (III) alkaline-degradate was prepared by mixing 50 mg of authentic (III) with 10 ml 0.1M sodium hydroxide, refluxing for 20.0 minutes, cooling, neutralizing with the media with 0.1M HCl, and raising the volume to 100 ml with phosphate buffer pH 7 to obtain a concentration of 0.5 $mg.ml^{-1}$.

Complete degradation is checked by TLC using silica gel 60 F254 plates and chloroform: ethyl acetate: methanol [8.0: 8.0: 4.0] as a developing system.

2-4. Procedures:

2-4.1.Spectrophotometric technique:

2-4.1.1. Determination of Trosemide:

First derivative of ratio spectra method (DR^1):

Calibration curve was performed by transferring aliquots of (I) working standard solution into a series of 25 ml volumetric flasks, and diluting to volume with 0.1M HCl to obtain a concentration range of 2–40 $\mu g.ml^{-1}$. The spectrum of acid-degradate solution having concentration 2.0 $\mu g.ml^{-1}$ was scanned and stored in the instrument PC as a devisor. The spectra of (I) were divided by the devisor's spectrum, then the first derivative of the ratio spectra (DR^1) were computed at 272.00 nm, plotted versus concentrations, and the regression equation was computed.

2-4.1.2. Determination of Irbesartan:

First derivative of ratio subtraction spectral method:

The overlapping spectra of a binary mixture, (II) with hydrochlorothiazide (HCTZ) were resolved by adopting the ratio subtraction technique. The spectra of (II) working standard solutions were scanned from 200–400 nm and stored in the computer. The spectra of the laboratory-prepared mixtures were divided (absorbance at each wavelength) by the spectrum of 10.0 $\mu g\ ml^{-1}$ of (HCTZ). The absorbance in the plateau region was subtracted at wavelength above 305 nm (the constant). The obtained curves were multiplied (absorbance at each wavelength) by the spectrum of 10.0 $\mu g\ ml^{-1}$ of (HCTZ). Then the first derivative of the ratio subtraction was computed at 262.00 nm, plotted versus concentrations, and the regression equation was computed.

2-4.1.3. Determination of Olmesartan:

2-4.1.3.1.A. First derivative of ratio spectra method (DR^1):

Into a series of 25 ml volumetric flasks aliquots of (III) working standard solution were transferred, and the volume was then diluted with phosphate buffer pH 7 to obtain a concentration range of 2–50 $\mu g.ml^{-1}$. The spectrum of alkaline-degradate solution having concentration 2.0 $\mu g.ml^{-1}$ was scanned and stored in the instrument PC as a devisor. The spectra of (III) were divided by the devisor's spectrum, then the first derivative of the ratio spectra (DR^1) were computed at 278.00 nm, the calibration curve was then plotted versus concentrations, and the regression equation was computed.

2-4.1.3.1.B. First derivative of ratio subtraction spectral method:

The spectra of (III) working standard solutions were scanned from 200–400 nm and stored in the computer. The spectra of the laboratory-prepared mixtures were divided (absorbance at each wavelength) by the spectrum of 5.0 $\mu g\ ml^{-1}$ of (HCTZ). The absorbance in the plateau region was subtracted at wavelength above 305 nm (the constant). The obtained curves were multiplied (absorbance at each wavelength) by the spectrum of 5.0 $\mu g\ ml^{-1}$ of (HCTZ). Then the first derivative of the ratio subtraction was computed at 268.00 nm, plotted versus concentrations, and the regression equation was computed, to resolve the overlapping present between the spectra of (III) and hydrochlorothiazide (HCTZ) binary mixture.

2-4.1.3.1.C. First derivative of pH-induced difference spectrophotometric method (DD^1):

Aliquots of (III) working standard solution were transferred into two sets of 25 ml volumetric flasks, diluted with borate buffer pH 8.0 in the first set and with 0.1M NaOH pH 13.0 in the second set, to obtain a concentration range of 2-40 $\mu g.ml^{-1}$. The absorption spectra of the first set were scanned against borate buffer pH 8.0 and the second set against 0.1M NaOH pH 13.0. The differences in the absorption spectra (ΔA) were determined and the first derivative of ΔA spectra (DD^1) was then computed. The calibration curve was constructed by plotting the amplitudes at 256.00 nm versus concentrations, and the regression equation was then computed.

2-4.2. Spectrofluorimetric technique:

This technique affords a higher sensitivity, if compared with those spectrophotometric and chromatographic ones, where it permits the determination of the examined substances in a concentration reaches to one part per trillion[60,61]. In this method, each of (I), (II) and (III) investigated drugs can be determined with a higher sensitivity.

2-4.2.1. Determination of Trosemide:

Aliquots equivalent to 0.3–1.5 ml of (I) working standard solution were transferred into 100.0 ml volumetric flasks and the volume was completed to the mark with 0.1M hydrochloric acid, to give a concentration of 300–1500 $ng.ml^{-1}$. The fluorescence intensity was recorded at $\lambda_{emission}$ 407 nm using $\lambda_{excitation}$ at 237 nm. The calibration graph was plotted representing the relationship between emission intensity against concentrations and the regression equation was computed.

2-4.2.2. Determination of Irbesartan:

The calibration curve was performed by transferring aliquots of (II) working standard solution into a series of 100 ml volumetric flasks, and diluting with water to obtain a concentration range of 300–2300 $ng.ml^{-1}$. The fluorescence intensity was recorded at $\lambda_{emission}$ 390 nm using $\lambda_{excitation}$ at 224 nm, which then plotted versus concentrations, and the regression equation was computed.

2-4.2.3. Determination of Olmesartan:

0.03–0.2 ml of (III) working standard solution was transferred into 100.0 ml volumetric flasks and the volume was completed to the mark with 0.1M hydrochloric acid, to give a concentration of 30–200 $ng.ml^{-1}$. The fluorescence intensity was recorded at $\lambda_{emission}$ 409 nm using $\lambda_{excitation}$ at 221 nm. The calibration graph was plotted representing the relationship between emission intensity against concentrations and the regression equation was computed.

2-4.3. Assay of the pharmaceutical preparations:

For spectrophotometric technique, twenty tablets of Examide®, Co-Approval®, Erastapex® and Erastapex plus® were individually weighed to get the average weight of the tablets and finely powdered, respectively. A sample of the powdered tablets, claimed to contain '50 mg' and '30 mg' of '(I) and (III)' and '(II) and (III)', was transferred separately to 100 ml volumetric flasks, dissolved in 50 ml of '0.1M HCl and methanol' for (I) and '(II) and (III)', filtered and then the volume was brought to 100 ml with the same solvents. Also, phosphate buffer pH 7 was used as a solvent for dissolving and diluting the powdered sample of (III) '50 mg' only, to be determined by adopting the derivative ratio technique. These prepared solutions were used as stock working solutions. While for spectrofluorimetric technique, a sample of the powdered tablets, claimed to contain '10 mg' of '(I), (II), and (III), were transferred separately to 100 ml volumetric flasks dissolved in 50 ml methanol, filtered and then the volume was brought to 100 ml with the same solvent to prepare stock working solutions. Then the mentioned procedure under 2.4., was utilized for both spectrophotometric and spectrofluorimetric methods.

2-4.4. Spectrofluorimetric Determination of Trosemide in plasma samples:

Into a 10 ml centrifuging-tube, aliquots equivalent to 20 and 30 µg of (I) working standard solution were transferred, followed by 1 ml of human plasma and vortexed for 20 second. Then 1.5 ml of acetonitrile was added to precipitate the proteins, vortexed for 30 second, followed by addition of 2 ml methanol, vortexed again for 1 min and then centrifuged at 3000 rpm for 20 min. The supernatant was transferred to 25-ml volumetric flask, evaporated to dryness at 70^0C under vacuum, then the residue was re-constituted with the least amount of methanol, vortexed for 20 second, and completed to the mark by 0.1M HCl. Then the relative fluorescence for each concentration was recorded, and the concentration was calculated from the regression equation. All the steps in this application was adopted according to CAROLINA et al[62].

3. Results and Discussion:

3.1.Spectrophotometric methods

The absorption spectra of (I) and (III) and their degradation products shown in (Figures 2a-2b), exhibit severe overlapping that prevents the use of direct spectrophotometric determination of each drug in presence of its degradate. So, derivative ratio was

utilized for determination of both investigated drugs in presence of their degradates. Also, pH-induced difference spectrophotometric technique was adopted for the determination of (III) in presence of its alkaline-degradate. The proposed scheme for degradation of (I) and (III) is shown in (Figures 3a-3b), where Fourier transform infrared "FT-IR" and mass spectrometry "MS" were used for explaining the degradation behavior of I and III.

The selection of the optimum wavelength was based on the fact that the absolute value of the total derivative spectrum at the selected wavelength has the best linear response to the analyte concentration. It is not affected by the concentration of any other component and gives a near-zero intercept on the ordinate axis of the calibration curve. Therefore, 272.0 nm and 278.0 nm were chosen as optimum working wavelengths for the determination of (I) and (III) in presence of their degradates by utilizing the proposed method, as shown in (Figures 4a-4b) respectively. Also 256.0 nm was used for determination of (III) in presence of its alkaline degradates by computing the first derivative of pH-induced difference spectrophotometry, as shown in (Figure 4c).

On the other hand, recently (II) and (III) were used separately in combination with HCTZ as antihypertensive drugs. Unfortunately, trails to determine either (II) or (III) in presence of HCTZ were not recorded, regarding to severe overlapping obtained in the absorption zero-order UV spectra of (II) and (III) and HCTZ, separately, as shown in (Figures5a-5-b). This extensive overlapping of the spectral bands of the two allowing us to utilized ratio subtraction technique, where 262.0 nm and 268.0 nm were selected as optimum working wavelengths for the determination of (II) and (III) in presence of HCTZ, as shown in (Figures 6a-6b) respectively.

3.2. Spectrofluorimetric method

A native strong fluorescence was observed upon dissolving '(I) and (III)' and (II) in 0.1M Hydrochloric acid and water, where these two solvents were selected among different solvents, including 0.1M hydrochloric acid, 0.1M sodium hydroxide, methanol and distilled water and the best emission intensity was obtained on using the last mentioned selected ones as dilution solvents for '(I) and (III)' and (II), as shown in (Figures 7a-7c), respectively. Scanning the emission spectra for the studied investigated drugs showed $\lambda_{emission}$ 407.0 nm, 409.0 nm and 390.0 nm, using $\lambda_{excitation}$ at 237.0 nm, 221.0 nm and 224.0 nm, attributing to the high conjugation, as shown in (Figures 8a-8c), respectively.

The adopted spectrofluorimetric method is highly sensitive, allowing us to determine (I) in plasma, where the recovery was found to be 92 %. In this application, different solvents were used to precipitate proteins including 6M hydrochloric acid [63], methanol [64] and acetonitrile[65, 66], but the best results were obtained in using acetonitrile, regarding to the disadvantage of methanol where incomplete precipitation of the plasma proteins was obtained, also abnormal brown color, which could be explained by the instability of the drug in acid media[67] in using hydrochloric acid. Consequently, the proposed precipitation and extraction method explained earlier was used[62].

3.3. Methods validation.

ICH-guidelines[4] for the methods of validation were followed, where all the validation parameters are shown in (Table 1).

3.3.1. Linearity:

A linear correlation was obtained between 'peak amplitude and/or fluorescence intensity' and concentration of the investigated drugs "I, II and III" in a range of '2-40 μgml^{-1}, 2-50 μgml^{-1} and (2-50, 2-40, 2-50 μgml^{-1})' with [correlation coefficient [r] = 0.9998, 0.9998 and '0.9998, 0.9997, 0.9997'] and '300–1500 ng ml^{-1}, 300–2300 $ngml^{-1}$ and 30–200 $ngml^{-1}$' with [correlation coefficient (r) = 0.9997, 0.9998 and 0.9998] for the spectrophotometric and spectrofluoremetric determinations, respectively.

3.3.2. Accuracy:

The accuracy of the proposed methods was tested by analyzing freshly prepared solutions of the studied drugs in triplicate. The recovery percent and standard deviations (S.D.) revealed excellent accuracy. The results obtained by applying the proposed methods were statistically compared with those results obtained by the reference methods [68-70]. It was concluded that with 95% confidence, there is no significant difference between them, since the calculated *t* and *F* values are less than the theoretical values [71].

3.3.3. Repeatability and reproducibility:

The intra- and inter-day precision was evaluated by assaying freshly prepared solutions in triplicate.

3.3.4. Specificity:

I and III were determined in solutions of laboratory prepared mixtures containing their acid and alkaline-degradates, also II and III could be determined in presence of HCTZ by the proposed

methods. The Recovery % and S.D. proved the high specificity of these methods, as shown in (Table 2).

3-4. Standard addition technique:

The proposed methods were applied for the determination of the studied drugs in the pharmaceutical preparations. The results were satisfactory and with good agreement with the labeled amount. Moreover, to check the validity of the adopted proposed methods, the standard addition method was applied by adding known amounts of the studied drugs to the previously analyzed tablets. The recoveries were calculated by comparing the concentration obtained from the spiked samples with that of each pure drug. The results of the commercial tablets analysis and the standard addition method (recovery study) of [I, II and III] are shown in (Tables 3-5) suggested that there is no interference from any excipients, which are normally present in tablets.

Also, the proposed adopted spectrofluorimetric method could be successfully applied for determination of I in spiked human plasma samples by liquid-liquid extraction technique, where the recovery was found to be 92 %, as shown in (Table 6).

3.5. Identification of Torasemide acid-degradate and Olmesartan medoxomil alkaline-degradate:

3.5.1. Identification of Torasemide acid-degradate

Structure elucidation of Torasemide acid-degradate exhibiting terminal amide bond cleavage, resulting in formation of hydroxyl and carbonyl groups, which was explained by utilizing FT-IR and M.S., techniques. In the FT-IR technique, the acid-degradate showed a similar absorption pattern to (I) except the appearance of the acid-degradate bands at 3463.4 and 1735.7 cm^{-1}, respectively, while in M.S., two peaks were delivered at m/z 59 and 307, respectively, (Figures 9a-9d).

3.5.2. Identification of Olmesartan alkaline-degradate

By the same manner, the structure elucidation of Olmesartan alkaline-degradate exhibiting ester bond cleavage, resulting in formation of hydroxyl and carbonyl groups, which was explained by utilizing FT-IR "Fourier transform spectroscopy" and M.S., techniques. In the FT-IR technique, the alkaline-degradate showed a similar absorption pattern to (III) except the disappearance of the ester carbonyl band at 1737.2 cm^{-1} and the appearance of the corresponding Hydroxyl and carbonyl bands of the carboxylic group of the degradation product at 3423.5 and 1712.7 cm^{-1}, respectively, on the other hand, mass spectrum of the alkaline degradation product exhibited two new peaks at m/z 130 and 446, respectively, (figures 10a-10d).

4- Conclusion:

The proposed methods were precise, specific, accurate and reproducible, where Torasemide, Irbesartan and Olmesartan can be determined in bulk powder and in pharmaceutical preparations without interference from excipients present, as well as in the presence of their different-degradates or other drug in-combination by the ICH-guidelines were followed throughout method validation and the suggested methods can be applied for routine quality control analysis and stability studies.

Table 1: Validation report of the proposed methods for determination of Torasemide (I), Irbesartan (II) and Olmesartan (III).

Parameters	Torasemide		Irbesartan		Olmesartan			
	Derivative Ratio	Spectrofluor-imetry	Ratio Subtraction	Spectrofluor-imetry	Derivative Ratio	Difference Spectrophotometry	Ratio Subtraction	Spectroflu-orimetry
	μgml^{-1}	$ngml^{-1}$	μgml^{-1}	$ngml^{-1}$	μgml^{-1}			$ngml^{-1}$
Linearity	2-40	300-1500	2-50	300-2300	2-50	2-40	2-50	30-200
Intercept	0.2682	0.0602	-0.001	0.4182	-0.0108	-0.0005	0.0293	-0.3923
Slope (b)[a]	0.2569	0.0685	0.0013	0.0452	0.0167	0.0012	0.001	0.537
Correlation Coefficient (r)	0.9998	0.9997	0.9998	0.9998	0.9998	0.9997	0.9997	0.9998
Accuracy[b]	100±0.57	100.23±0.9	100.02±0.92	100.86±1.24	100.08±0.73	99.95±0.79	101±0.47	99.91±1.12
Precision								
Repeatability[b]	104±0.57	99.6±0.47	99.6±0.45	99.8±0.66	100.2±0.45	100.2±0.25	99.8±0.48	100.1±0.65
Intermediate Precision[b]	99.8±0.64	99.8±0.63	99.5±0.68	99.6±0.78	99.8±0.74	100.4±0.61	100.1±0.61	100.4±0.74

[a]Regression equation = "A = a + bc". [b]Mean ± S.D.

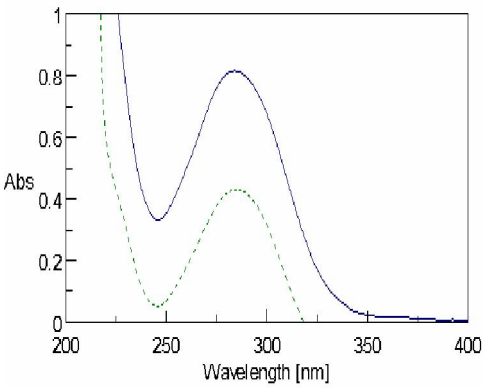

Figure (2-a): Zero-order absorption spectra of Torasemide (—) and its acid-degradate (...), (each of 20.0 μg ml^{-1}).

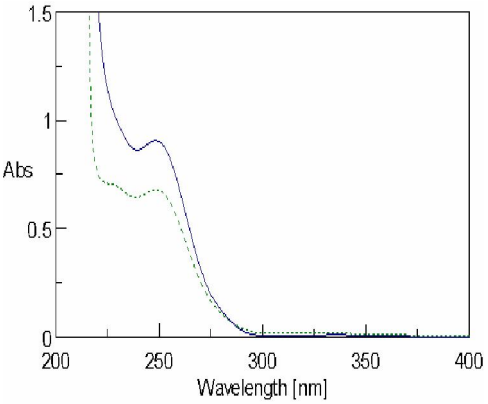

Figure (2-b): Zero-order absorption spectra of Olmesartan (—) and its alkaline-degradate (...), (each of 20.0 μg ml^{-1}).

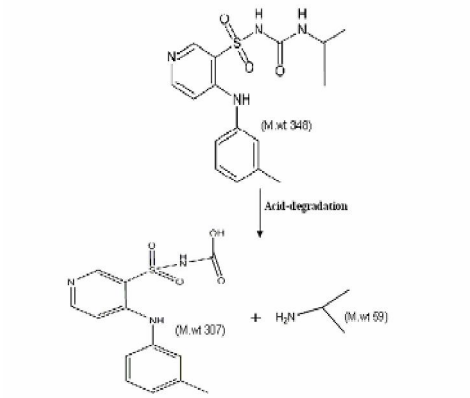

Figure (3-a): Suggested degradation pathway for Torasemide.

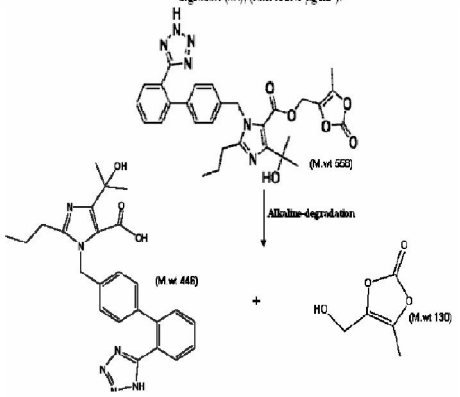

Figure (3-b): Suggested degradation pathway for Olmesartan.

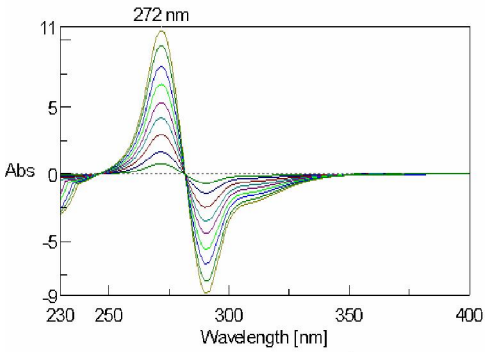

Figure (4-a) First-derivative of the ratio spectra (DR^1) for different concentrations (2.0-40.0 μg ml^{-1}) of Torasemide at 272.0 nm, using 2.0 μg ml^{-1} of its acid-degradate as a devisor.

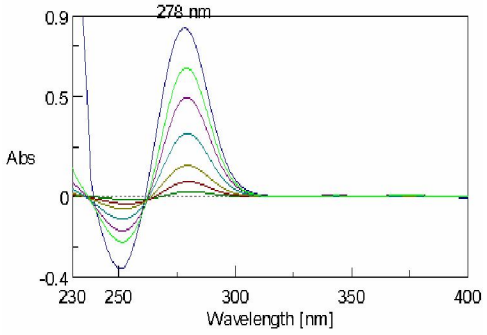

Figure (4-b): First-derivative of the ratio spectra (DR^1) for different concentrations (2.0-50.0 μg ml^{-1}) of Olmesartan at 278.0 nm, using 2.0 μg ml^{-1} of its alkaline-degradate as a devisor.

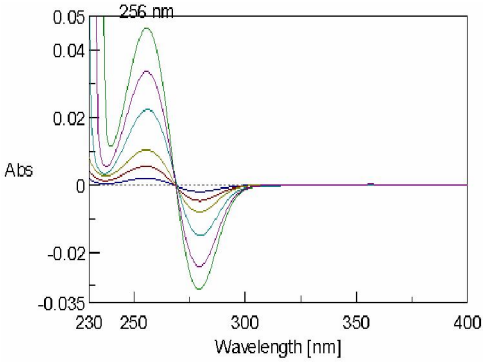

Figure (4-c): First derivative of difference spectra (DD^1) for different concentrations (2.0–40.0 $\mu g ml^{-1}$) of Olmesartan medoxomil at 256.0 nm.

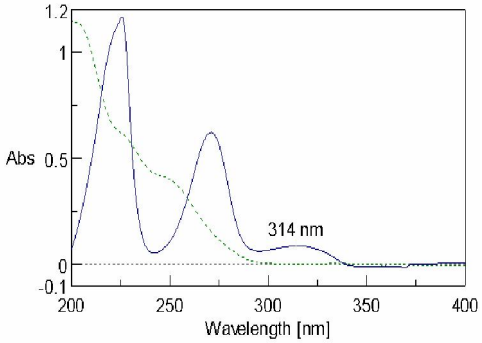

Figure (5-a): Zero-order absorption spectra of Irbesartan (....) and HCTZ (—), (each of 10 $\mu g ml^{-1}$).

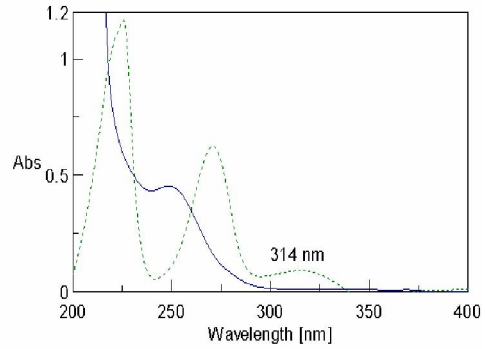

Figure (5-b) Zero-order absorption spectra of Olmesartan (—) and HCTZ (....), (each of 10 $\mu g ml^{-1}$).

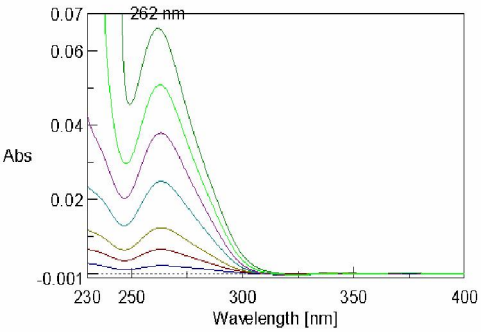

Figure (6-a): First order division spectra of laboratory-prepared mixtures of Irbesartan and HCTZ, at 262.0 nm, using 10 $\mu g ml^{-1}$ of HCTZ as the divisor.

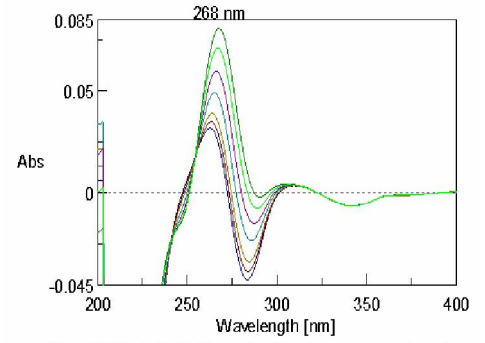

Figure (6-b): First order Division spectra of laboratory-prepared mixtures of Olmesartan and HCTZ, at 268.0 nm, using 5 $\mu g ml^{-1}$ of HCTZ as the divisor.

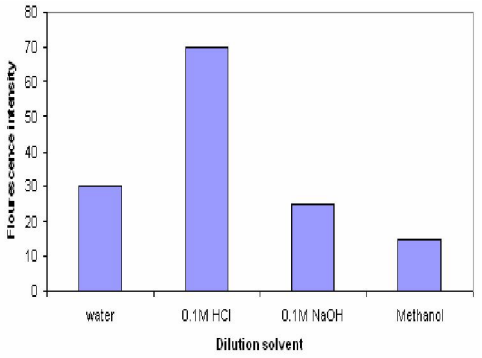

Figure (7-a): Effect of dilution solvent on fluorescence intensity of Torasemide in a concentration (1 $\mu g ml^{-1}$).

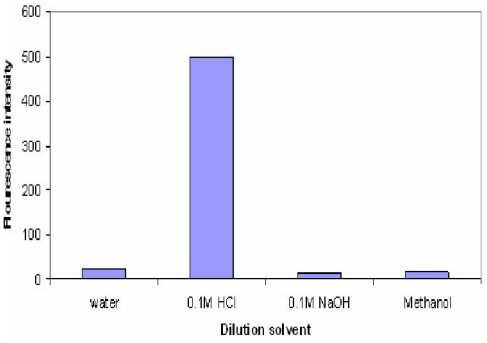

Figure (7-b): Effect of dilution solvents on fluorescence intensity of Olmesartan in a concentration (1 $\mu g ml^{-1}$).

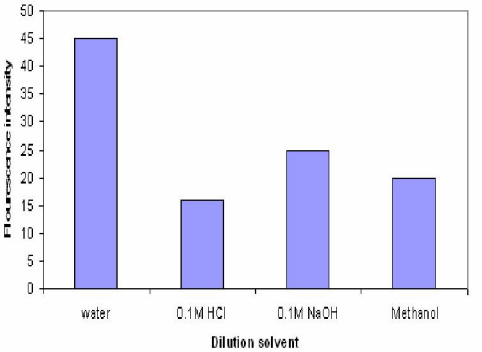

Figure (7-c): Effect of dilution solvents on the fluorescence intensity of Irbesartan in a concentration (1 $\mu g ml^{-1}$).

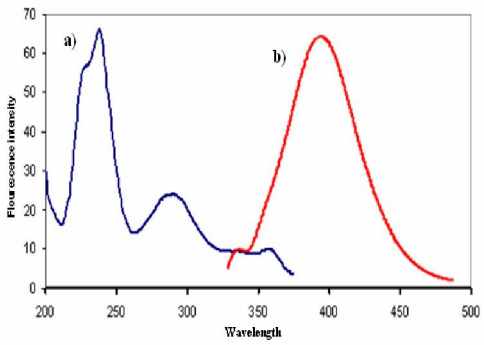

Figure (8-a): Excitation (a) and Emission (b) spectra of Torasemide in 0.1 M HCl

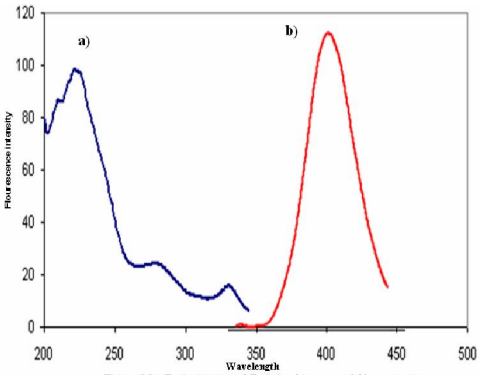

Figure (8-b): Excitation (a) and Emission (b) spectra of Olmesartan in 0.1M HCl

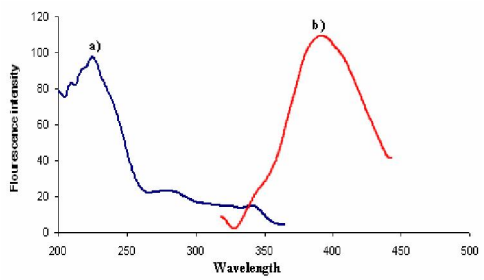

Figure (8-c): Excitation (a) and Emission (b) spectra of Irbesartan in Distilled water.

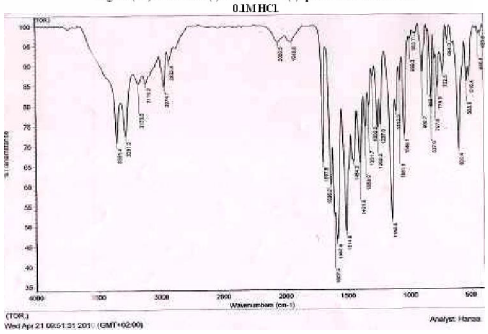

Figure (9-a): IR spectrum of the Intact Torasemide.

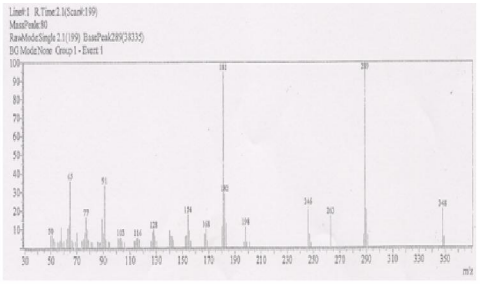

Figure (9-b): Mass spectrum of the intact Torasemide.

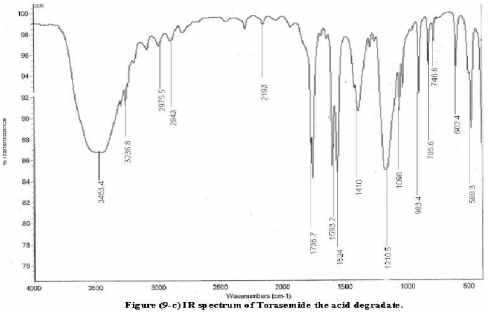

Figure (9-c) IR spectrum of Torasemide the acid degradate.

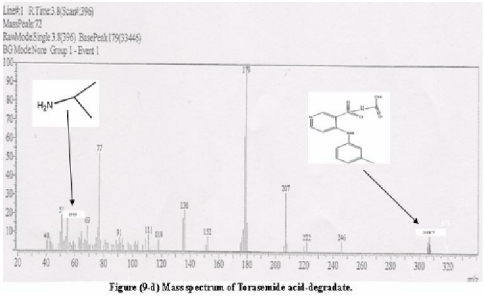

Figure (9-d) Mass spectrum of Torasemide acid-degradate.

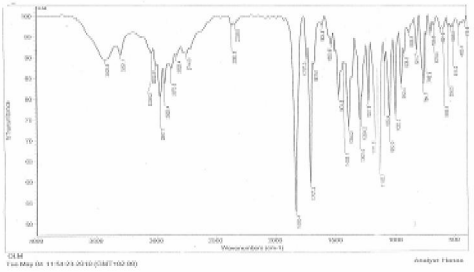

Figure (10-a): IR spectrum of the Intact Olmesartan.

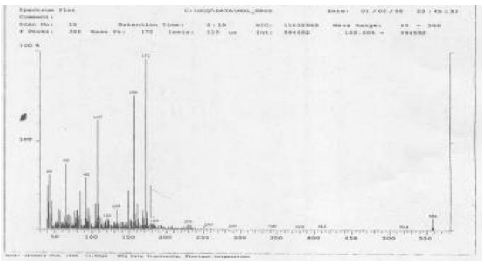

Figure (10-b): Mass spectrum of the intact Olmesartan.

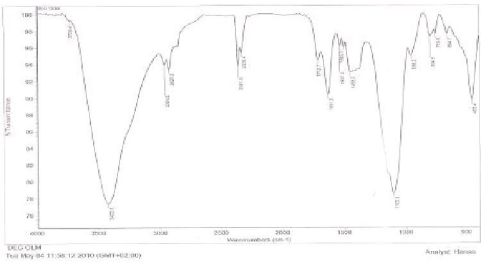

Figure (10-c): IR spectrum of Olmesartan alkaline degradate.

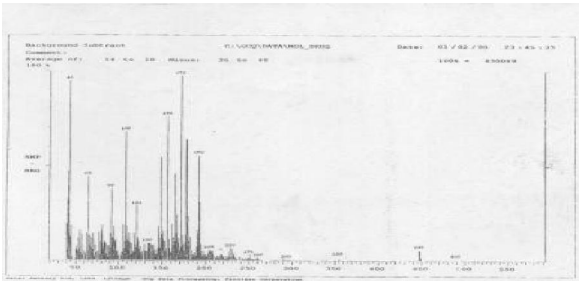

Figure (10-d): Mass spectrum of Olmesartan the alkaline degradate.

Table 2: Results for Torasemide (I), Irbesartan (II) and Olmesartan (III) in laboratory prepared mixtures by the proposed methods:

Sample No.	% of Interfering substance	Recovery%*						
		Torasemide	Irbesartan		Olmesartan			
		Derivative Ratio	Ratio Subtraction	Spectrofluor-imetry	Derivative Ratio	Difference Spectrophotometry	Ratio Subtraction	Spectroflu-orimetry
1	20	100.5	101.54	100.16	100.48	100.83	99.30	99.43
2	30	98.55	100.77	-	101.68	99.67	100.00	-
3	40	99.72	100.38	100.53	98.68	100.00	99.90	101.29
4	50	98.55	99.23	-	101.08	100.75	101.00	-
5	60	101.67	99.92	100.90	100.48	101.17	100.80	101.66
6	70	101.28	100.77	-	100.78	102.00	101.15	-
7	80	-	101.54	98.69	101.08	-	-	98.68
8	90	-	102.15	99.10	100.78	-	-	101.29
9	100	-	-	101.64	101.38	-	-	100.36
Mean		100.04	100.79	100.17	100.93	100.74	100.42	100.45
S.D.		1.34	0.95	1.11	0.31	0.83	0.82	1.19

*Mean of four determinations.

Table 3: Determination of Torasemide in pharmaceutical preparation[a] by the proposed {spectrophotometric and spectrofluorimetric} methods and application of standard addition technique.

Pharmaceutical Preparation	Claimed	% Found ± SD*		Standard addition technique							
		Derivative Ratio	Spectro-fluorimetry	Derivative Ratio	Spectro-fluorimetry	Pure added		Pure found		Recovery %*	
				Taken in μgml^{-1}	Taken in $ngml^{-1}$	Derivative Ratio in μgml^{-1}	Spectro-fluorimetry in $ngml^{-1}$	Derivative Ratio in μgml^{-1}	Spectro-fluorimetry in $ngml^{-1}$	Derivative Ratio	Spectro-fluorimetry
Examide ® tablets 20 mg B.N: MT1120410[a]	20 mg	99.2 ±0.54	101.5 ±0.46	10	300	2	50	1.996	50.60	99.80	101.20
						5	100	5	99.40	100.00	99.40
						15	150	15.075	149.780	100.50	99.80
						20	200	20.04	201.00	100.20	100.50
						25	250	24.925	253.00	99.70	101.20
						30	300	30.06	298.80	100.20	99.60
Mean ±S.D.										100.07 ±0.29	100.28 ±0.80

*Mean of four separate determinations.

Table 4: Determination of Irbesartan in pharmaceutical preparation[a] by the proposed {spectrophotometric and spectrofluorimetric} methods and application of standard addition technique.

Pharmaceutical Preparation	Claimed	% Found ± SD*		Standard addition technique							
		Ratio Subtraction	Spectro-fluorimetry	Ratio Subtraction	Spectro-fluorimetry	Pure added		Pure found		Recovery %*	
				Taken in μgml^{-1}	Taken in $ngml^{-1}$	Derivative Ratio in μgml^{-1}	Spectro-fluorimetry in $ngml^{-1}$	Derivative Ratio in μgml^{-1}	Spectro-fluorimetry in $ngml^{-1}$	Ratio Subtraction	Spectro-fluorimetry
Co-Approval® tablets 300mg/12.5mg B.N: 1145[a]	300 mg	100.20 ±0.55	98.50 ±0.47	10	300	2	50	2.002	49.90	100.10	99.80
						5	75	4.945	75.00	98.90	100.00
						10	100	9.960	100.50	99.60	100.50
						20	125	20.040	125.25	100.20	100.20
						30	150	30.030	149.55	100.10	99.70
						40	200	40.080	200.40	100.20	100.20
Mean ±S.D.										98.85 ±0.52	100.07 ±0.29

*Mean of four separate determinations.

Table 5-a: Determination of Olmesartan in pharmaceutical preparation[a] by the proposed derivative ratio and pH-induced difference spectrophotometric methods [DR^n and DD^n] and application of standard addition technique.

Pharmaceutical Preparation	Claimed	% Found ± SD*		Standard addition technique							
		DR^n	DD^n	Taken μgml^{-1}		Pure added μgml^{-1}		Pure found μgml^{-1}		Recovery %*	
				DR^n	DD^n	DR^n	DD^n	DR^n	DD^n	DR^n	DD^n
Erastapex® tablets 40 mg B.N: MT 3241009[a]	20 mg	100.1 0 ±0.42	100.50 ±0.52	10	10	5	5	4.99	5.01	99.80	100.20
						10	10	10.02	10.01	100.20	100.10
						20	15	19.84	14.97	99.20	99.80
						25	20	25.15	20.06	100.60	100.30
						35	25	35.035	25.30	100.10	101.20
						40	30	40.08	29.94	100.20	99.80
Mean ±S.D.										100.02 ±0.48	100.23 ±0.52

*Mean of four separate determinations.

Table 5-b: Determination of Olmesartan in pharmaceutical preparation[a] by the proposed {spectrophotometric and spectrofluorimetric} methods and application of standard addition technique.

Pharmaceutical Preparation	Claimed	% Found ± SD*		Standard addition technique							
		Ratio Subtraction	Spectro-fluorimetry	Ratio Subtraction	Spectro-fluorimetry	Pure added		Pure found		Recovery %*	
				Taken in μgml^{-1}	Taken in $ngml^{-1}$	Derivative Ratio in μgml^{-1}	Spectro-fluorimetry in $ngml^{-1}$	Derivative Ratio in μgml^{-1}	Spectro-fluorimetry in $ngml^{-1}$	Ratio Subtraction	Spectro-fluorimetry
Erastapex plus® tablets 40mg/12.5mg B.N: MT0280110[a]	40 mg	100.20 ±0.48	98.80 ±0.84	10	300	5	20	4.99	20.02	99.80	100.10
						10	30	10.00	29.67	100.00	98.90
						20	50	20.10	49.80	100.50	99.60
						25	60	25.05	60.12	100.20	100.20
						35	80	34.895	80.08	99.70	100.10
						40	100	40.08	100.20	100.20	100.20
Mean ±S.D.										100.07 ±0.29	99.85 ±0.52

*Mean of four separate determinations.

Table 6: Determination of Torasemide in spiked human plasma by the proposed spectrofluorimetric method.

Spiked concentration ($ngml^{-1}$)	Recovery % ± S.D*
800.00	92.27 ± 0.56
1200.00	92.31 ± 0.47

* The mean percentage recovery of 3-separate determinations.

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Repair Welding Restoration of the Screw Conveyor for Resin Extruder

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Abstract: A screw conveyor was exposed to an extensive wear at the top and the side surfaces of the teeth. The microstructure of the base metal is martensitic structure. Welding procedure specification (WPS) and Process Qualification Record (PQR) were carefully performed using a scraped part from the screw conveyor. The preheating temperature of 300 to 400 °C was applied and the SMAW process was selected as a welding process. Three types of electrodes were selected which mainly wear and corrosion resistance type. Using chromium Carbide electrodes resulted in a significant appearance of cracks at the weld surface that extended to the heat affected zone. However, Using martensitic electrodes resulted in a crack free weld metal with a significant improve of the wear resistance of the base metal. The effect of applying cushion layer between the base metal and hardfacing layer were studied using two kinds of covered electrodes. The hair cracks that observed using the hardfacing electrodes were greatly reduced using these cushion layers. The results were discussed on the basis of microstructure and the wear resistance of the base metal and the hardfacing layers.

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Keywords: Welding restoration; Screw Conveyor; Hardfacing electrode; Cushion layer; Microstructure; Wear index

1. Introduction:

Engineers in many industries are concerned with prolonged the life of the structure, with the repair and reclamation of its worn surface on its broken components (1). Fortunately, there are many cases where the extent of damage or worn is small and localized which enables the component to be repaired economically by welding (1).

Weld surfacing is a useful tool for restoration of worn industrial components. The wide range of consumables available for use with the many welding processes requires careful selection to suit a given working environment (2).

Restoration is indicated when welding is done at a low cost compared with replacement costs especially when the component is large and/or expensive or is a part of a larger structure.

Having suitable welding procedures and fulfilling the metallurgical requirements are the first two vital factors for successful repair (1, 3).

The aim of the research work is to study the repair weldability of the screw conveyor of resin extruder. The results were discussed on the basis of microstructure, hardness, wear index of the base metal and the hardfacing layers and the occurrence of cracks.

2. Materials and Methods:

2.1 Materials

A part of the screw conveyor was used as a base metal. Specification and chemical composition of the base metal is shown in Table 1. Figure 1 shows the complete structure of the extruder and the location of the pair of screw conveyor. The tooth outer surface is exposed to mainly abrasion wear and their diameter was decreased by 10 mm. Also, the side part of the tooth was exposed to worn especially at the mid length of the screw conveyor.

Three types of special SMAW electrodes were applied in the repair welding process. Also, stainless steel and mild steel electrode are applied as a cushion layer between the base metal and the special weld metals. Table 2 shows the specification of these electrodes. The chemical analysis was carried out using optical emission spectrometry instrument.

Table 1 Chemical composition of the screw conveyor, mass %

C	Si	Mn	Ni	Cr	Mo	S	P	Cu
0.6	0.26	0.71	1.89	1.02	0.46	0.003	0.001	0.13

Table 2 specification of welding electrodes applied in repair welding process

Electrode no	specification	
	AWSA 5.1	DIN:8555
A	E7016	E6-UM-60
B		E10-UM-60
C		E10-UM-60
D		
E		E8-UM-200KRZ

Fig. 1 Complete structure of the extruder and the location of the screw conveyor

2.2 Microstructures of base and deposited metals

Teeth base metal and the hard-faced samples were cut out using cooling disk machine. The cross sections were then ground through grit silicon papers (from 180 to 1000). Final polishing was performed using 0.5µm-alumina past, then cleaned and dried. Microstructures of welded specimens were observed, using Nickon-Epiphot optical microscope.

2.3 Hardness distribution

Hardness test was conducted using DVK-2 Matsuzawa Vickers hardness testing device at a load of 20 kgf. for 15 second loading time, and 70 µm/s load speed.

2.4 Abrasion resistance test

Abrasion wear test pin-on disc, for the weld deposits and base metal was conducted using Tribometer testing machine. All tests were carried out under pure sliding conditions between specimens and abrasive disc (alumina abrasive particles). The abrasion test sample with 8 mm diameter and 11 mm length was machined from the welded specimen, so that the test surface was parallel to the layers of the deposits. The test surfaces were flattened by grinding in order to remove the curvature of the weld bead deposit. The test specimens were cleaned in acetone using ultrasonic, before and after test, then weighted to four decimals. The rotation speed of abrasive disc is 125 rpm with a load of 70 N and for 10 minutes. An abrasive disc of 73 mm diameter and 60 mm mesh size alumina particles is used. Wear index is calculated by dividing the weight loss of the deposited metal of the electrode to the weight loss obtained using the base metal.

3. Results and Discussion:

3.1 Welding procedure specification

Welding procedure specifications were conducted using three different SMAW electrodes, namely A, B and C electrodes as shown in Table 2. Preheating temperature ranged from 300 to 400 0C was applied and the interpass temperature was also adjusted to the same preheat-temperature range. Stringer bead technique was used in depositing the beads at the two edges of the teeth. However, between these two stringer beads, weaving tight bead technique was applied to cover the entire bead surface with minimum dilution. After completion of the first layer, grinding was applied to remove the convexity of the layer surface and also to reduce the residual stresses (half bead technique). By this way, welding was proceeded until making five layers sufficient to restore the original diameter of the screw conveyor.

Application of five layers of electrode C resulted in a formation of transverse surface cracks as detected by dye-penetrant test (Fig. 2a). The same results were obtained with the use of electrode B. However, the crack density significantly decreased than that obtained using electrode C as shown in Fig. 2b. A crack free- weld was obtained with the use of electrode A as shown in Fig. 3 a.

Welding of the whole screw conveyor was conducted with the same welding procedure using electrode A. Dye-penetrant test shows a few transverse surface cracks distributed along each teeth surface. These cracks are extended to the heat affected zone as shown in Fig. 5 b. This could be attributed to the accumulated thermal stresses and the constraint associated with the welding of the whole screw conveyor.

3.2 Effect of cushion layer application

The effect of application of a cushion layer using electrode D and E (Table 2) between the base metal and the hardfacing layers on the soundness of weldment was also studied. Using cushion layers of electrode D (Fig. 3b) or electrode E (Fig. 3c) between the base metal and the hardfacing layers (three layers of electrode B) resulted in a significant decrease in the density of transverse cracks than that obtained without application of cushion layer (Fig. 2c).

Application of cushion layer using electrode E between the base metal and the hardfacing layers of electrode A in welding of the whole screw conveyor resulted in the disappearance of the transverse cracks and a sound weld was obtained as detected by dye-penetrant and ultrasonic inspections.

3.3 Chemical composition of deposited layer

The chemical compositions at the top fifth layer using electrodes A, B and C are given in Table 3. Deposited metal obtained using electrode A has the lowest carbon and chromium content. Electrode B has a higher carbon and chromium content than that obtained using electrode A. Electrode C has the highest carbon and chromium content. Electrode B has a higher Mn, Si, and Mo content than that obtained using electrode A or C. However, electrode A has a higher V content than that of electrode B or C.

The application of a cushion layer of electrode E or D resulted in a significant decrease in the carbon and chromium content of the weld deposited by electrode A and a slight decrease in the carbon and chromium content of the weld deposited using electrode B. Moreover, application of cushion layer using electrode E (stainless steel electrode) resulted in a recovery of some Ni and Mn in the hardfaced layer using electrode A or B as shown in Table 3.

3.4 Microstructure of base metal and weld deposits

The microstructure at the cross section of the teeth of the screw conveyor is shown in Fig. 4. It is martensitic structure.

The microstructure of the heat affected zone in the crack free specimen is shown in Fig. 5a. The microstructure reveals the existence of coarse martensite. The microstructure of the heat affected zone in the cracked specimen is shown in Fig. 5b. It is also a coarse martensite.

The microstructure at the top surface of the weld metals using electrode A, B and C are shown in Fig. 6a, b and c respectively. The microstructure of the weld metal using electrode A reveal the martensitic structure (Fig. 6a). The microstructure of the weld metal obtained using electrode B shows austenite with eutectic carbide (Fig. 6b). Weld metal microstructure obtained using electrode C shows the existence of Cr-carbides with austenite eutectic carbide matrix (Fig. 6c). The chromium carbide using electrode C is much coarser than that obtained using electrode B. It is already decided that the coarser the carbide the higher the wear resistance of the hardfacing layer (4).

3.5 Hardness distribution

Hardness distribution through the cross section of weld using five layers of electrode A is shown in Fig. 7. The hardness values increased from the base metal to the heat affected zone and then gradually increased to near 700 Hv20. The hardness distribution through the cross section of the weld using two layers of electrode E followed by three layers of electrode A is shown in Fig. 8. The hardness value increased from the base metal to the heat affected zone (more than 500 Hv20) and then decreased to more than 200 Hv and then increased gradually to more than 500 Hv20.

Microhardness values of chromium carbides and eutectic matrix at the top layer using 5 layers of electrode B and C are shown in Table 4. The microhardness values using electrode C is a little higher than that obtained using electrode B.

3.6 Abrasion index of base metal and weld deposits

The wear index of the base metal and the different weld deposits are shown in Fig. 9. The wear resistance of all the weld deposits is much better than that of the base metal. Wear resistance of electrode B and C deposits is much higher than that of electrode A deposit.

The higher the difference in hardness between the abrasive particles and the hard phases in the matrix of microstructure the lower the wear resistance and the higher the wear index value (Fig. 9). The microhardness of the alumina particle is about 2100 Hv (5).

However, the cracks appeared on the surface and HAZ while using electrode B and C preclude their usage in the repair welding of the screw conveyor.

From the foregoing results, it is preferred to apply electrode A (martensitic structure) since its deposit has both the characteristics of crack free and high wear resistance. The use of cushion layer before applying electrode, A resulted in a decrease in the wear resistance as shown in Fig. 9. This obviously appears when the pair of screw conveyor was put into service.

4. Conclusion:

A low alloy steel screw conveyor was exposed to an extensive wear at the top and the side surfaces of the teeth. Welding procedure specification (WPS) and Process Qualification Record (PQR) were carefully performed using a scraped part from the screw conveyor in order to repair reclamation of the screw conveyor. The following results were obtained:

1. Using chromium Carbide electrodes resulted in a significant appearance of cracks at the weld surface that extended to the heat affected zone. However, Using martensitic electrodes resulted in a crack free

weld metal with a significant improve of the wear resistance of the base metal.

2. The effect of applying cushion layer between the base metal and hardfacing layer were studied using two kinds of covered electrodes. The hair cracks that observed using the hardfacing electrodes were greatly reduced using these cushion layers.

3. Macro hardness is not an accurate measure of the wear resistance; however, microhardness can give an accurate one.

4. It is preferred to apply electrode a (martensitic structure) in repair welding of worn teeth of the screw conveyor since its deposit has both the characteristics of crack free and high wear resistance. The use of cushion layer before applying electrode, A resulted in a decrease in the wear resistance with a high crack resistance. Moreover, the wear resistance is still higher than that of the screw conveyor material.

Table 3 Chemical composition at the top surface of deposited layers, %

Welding sequence	C	Si	Mn	P	S	Cr	Mo	Ni	V
5 layer A	0.50	0.7	0.36	0.01	0.001	8.4	0.46	0.2	0.57
2 layers E & 3 layers A	0.42	0.49	0.28	0.01	0.001	5.2	0.35	1.8	0.29
2 layers D & 3 layers A	0.43	0.43	0.29	0.01	0.002	5.1	0.29	0.13	0.3
5 layers B	2.49	1.05	1.05	0.03	0.002	26.48	0.95	0.26	0.34
2 layers E & 3 layers B	2.23	0.92	1.47	0.025	0	24.41	0.63	1.58	0.299
2 layers D & 3 layers B	2.3	0.92	0.98	0.03	0.002	23.49	0.86	0.28	0.316
5 layers of C	5.12	0.68	0.412	0.01	0.001	34.8	0.036	0.036	0.03

Table 4. Microhardness of matrix and carbides using electrodes B and C

Electrode type	Microhardness Hv 200gm	
B	700	1200
C	730	1600

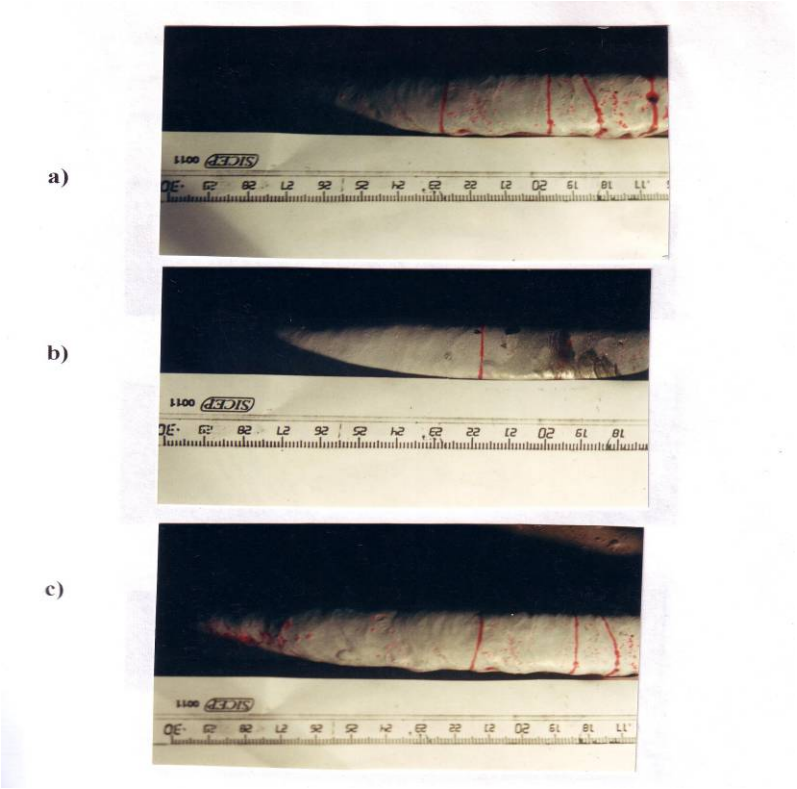

Fig. 2 Transverse surface cracks by dye penetrant test

a) Using electrode C (five layers),

b) Using a cushion layer E and 2layers of electrode C

c) Using electrode B (five layers).

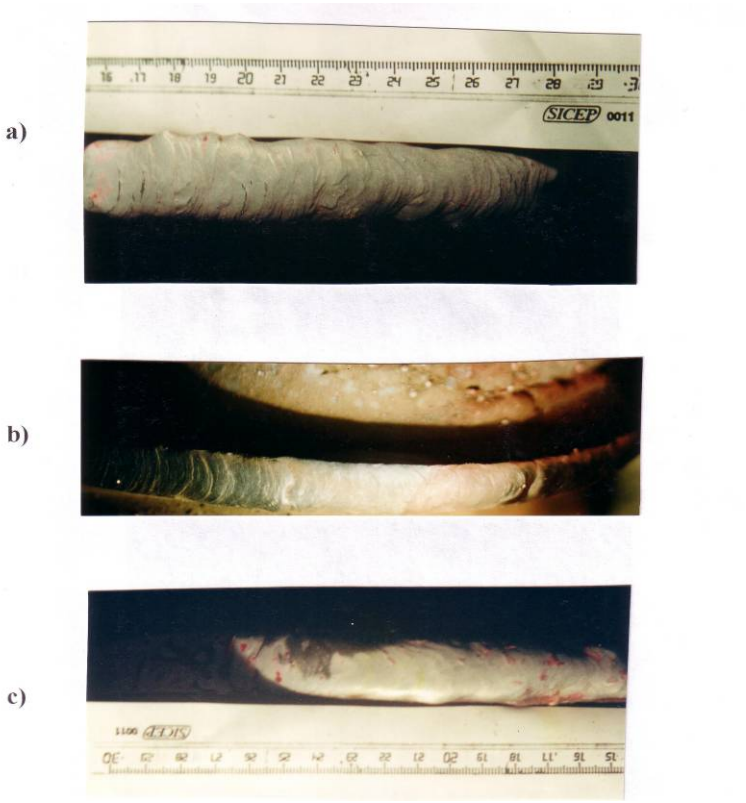

Fig 3 weld beads free from cracks as detected by dye penetrant test

a) Using electrode A (five layers),
b) Using a cushion layer D and 2 layers of electrode B
c) Using a cushion layer E and 2 layers of electrode B

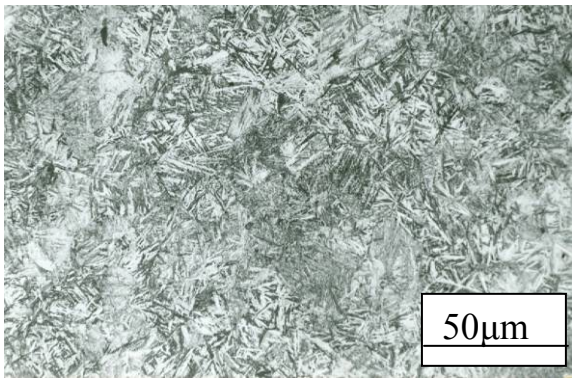

Fig. 4 Microstructure of the screw conveyor

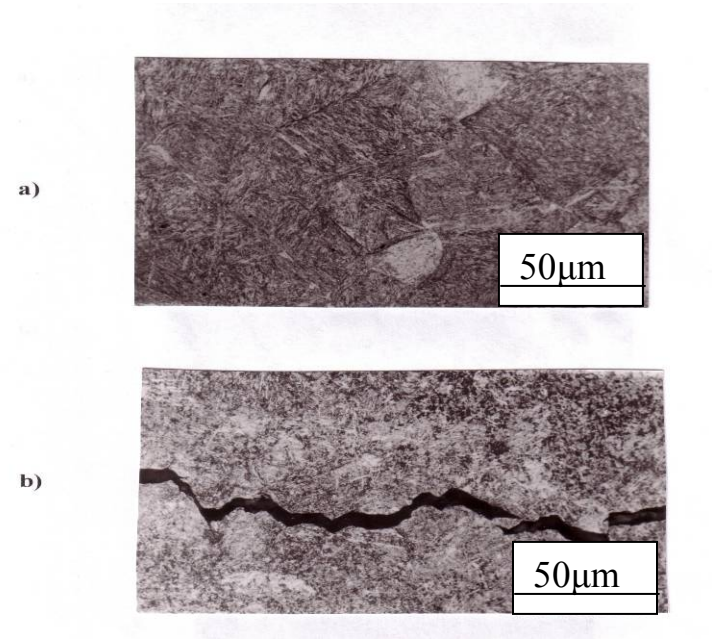

Fig. 5 Microstructure at the HAZ of the base metal a) Crack free specimen,

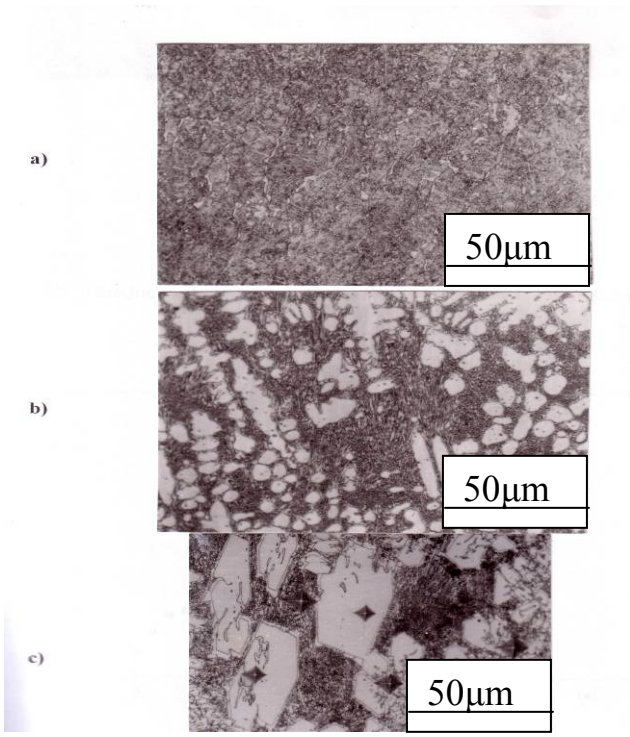

Fig. 6 Microstructure at the top surface of the weld metal using a) Electrode A, b) Electrode B, and Electrode C

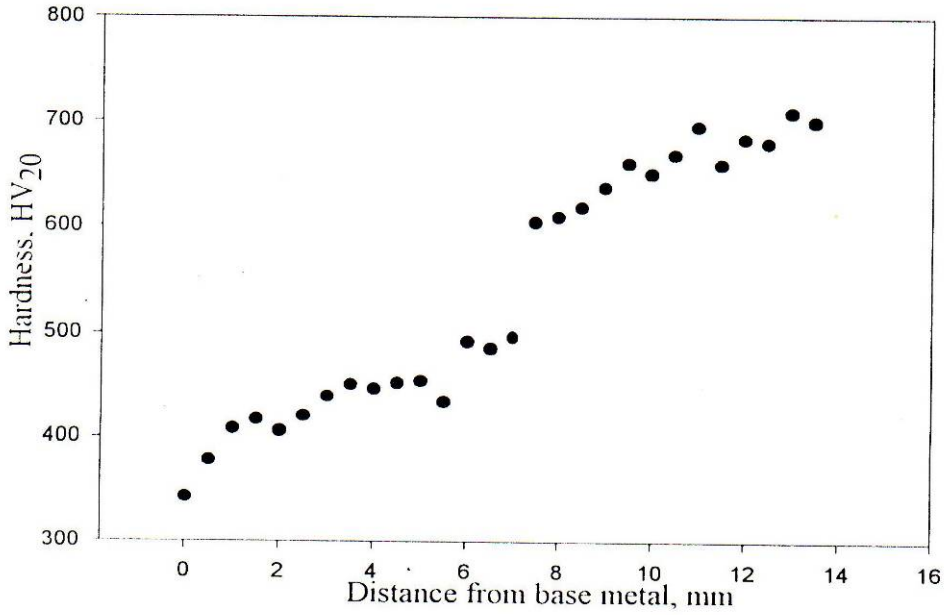

Fig. 7 Hardness distribution along the base metal, HAZ and hardfacing material using electrode A

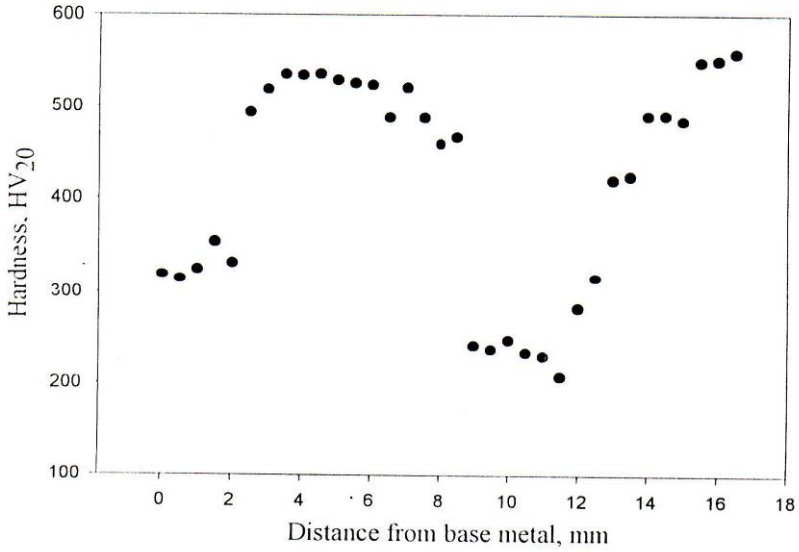

Fig. 8 Hardness distribution along the base metal, HAZ and hardfacing material using electrode E and three layer of electrode A.

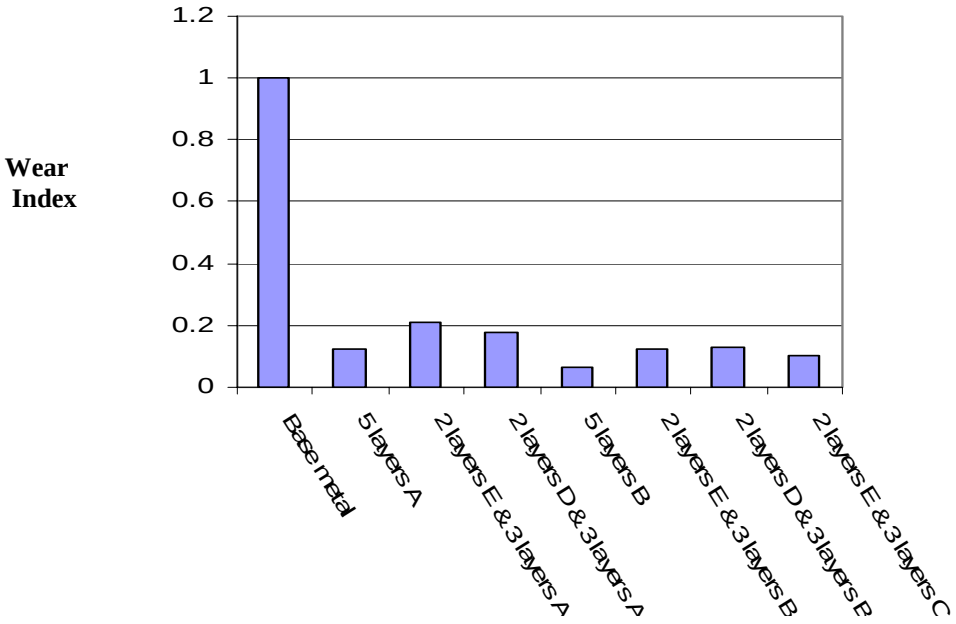

Fig. 9 Wear index for base metal and hardfacing layers

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Assessment of Farmers Knowledge Regarding Innovation Management in Farming Cooperatives in Shoushtar Township, Iran

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Abstract: The purpose of research is assessment of farmer's knowledge regarding innovation management in farming cooperatives in Shoushtar township of Khouzestan province, Iran. The method of research was correlative descriptive and causal relation. A random sample of Shoushtar township farmers of Khouzestan province, (n=105) were selected for participation in the study. According to results knowledge of farmers regarding management of innovation was moderate. Also regression showed that accessing to communication channel, level of education, income, crop yield, size of farm, social participation, level of participation in extension classes may well explain for 53% (R^2=0.534) changes in knowledge of farmers regarding management of innovation.
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Keywords: Innovation Management, Farmers, Shoushtar

1. Introduction

A cooperative is a group of individuals who take interactive profit from the coordination of production decision, mutual access to inputs, including seed, improved market power, and more effective lobbying capacity. The cooperative can acquire information about seeds and crops acquire the seed, process the crop, or market the product in order to create profit and to scatter the gains to members. (Falco, Smale & Perrings, 2007).

Fundamentally, agricultural cooperatives are user-owned and user-monitored movement, they return extra income, they are stimulated members by supplying a service to satisfy members' requirement for affordable and degree of excellence goods and they are self-reliance, self- responsibility, self – assistance and autonomous. (Doyer, 2005)

Not only agricultural cooperatives have several advantages such as open membership, democratic control and continuous education, But also this individually owned business has some disadvantages such as any losses borne by the owner be shared and capital limits the size of the business.(Birchall, 2005)

Innovation in business has been studied by multiple researchers (Kleefl, 2007., Kotelinkov, 2008., Kwamena, 2008).

Innovation allowing companies and economies to stay competitive in ever changing world markets. For all of the talk about the importance of innovation, innovation management and creativity in business, the topics are hardly generally well understood (Riederer et al, 2005).

Innovations management uses the systems and business to make the organization more innovative. The aim of innovations management is to maintain and improve the competitive position of the business by usage of innovation. The purpose of this research is assessment of farmer's knowledge regarding innovation management in farming cooperatives in Shoushtar township of Khouzestan province, Iran. Also at this research used one framework with subsystems of innovations management and analyzed linkage to other variables (Figure 1).

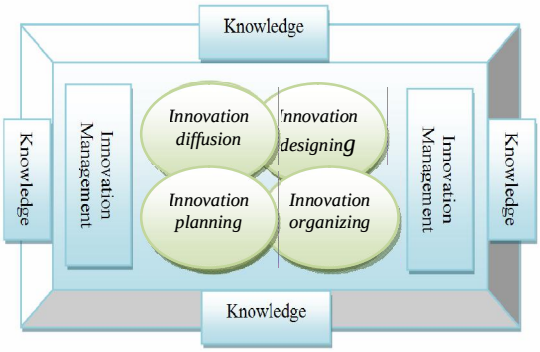

Figure 1: Theoretical Framework of Research

2. Material and Methods

The method of research was correlative descriptive and causal relation. A random sample of Shoushtar township farmers of Khouzestan province, Iran (n=105) were selected for participation in the study. A questionnaire was developed to gather farmer's knowledge regarding innovation management in farming cooperatives.

The questionnaire was pilot tested in Dezful township. Questionnaire reliability was estimated by

calculating Cronbach's alpha. Reliability was (Cronbach's alpha=0.87).Data collected were analyzed using the Statistical Package for the Social Sciences (SPSS). Appropriate statistical procedures for description (frequencies, per cent, means, and standard deviations) were used.

3. Results

For assessment of farmer's knowledge regarding innovation management in farming cooperatives were used 4 subsystems of innovation management and farmer's knowledge regarding each item in Likert domain analyzed.

KIDe=Knowledge Regarding Innovation designing
KIP= Knowledge Regarding Innovation planning
KIO= Knowledge Regarding Innovation organizing
KIDi= Knowledge Regarding Innovation diffusion

According to results, 51% of farmers had moderate knowledge regarding innovation management (Table 1). Also mean rank and standard deviation of farmer's knowledge include: M1=2.653, sd1=1.08, M2=2.091, sd2=1.01, M3=2.761, sd3=0.93, M4=3.112, sd4=1.04 (Table 2).

Also to identify the correlation between selected independent variables with the dependent variable (farmer's knowledge regarding innovation management). In this study, there was a significant relationship between the farmer's knowledge regarding innovation management with accessing to communications channels, level of education, income, crop yield, size of farm, social participation, and level of participation in extension classes (Table 3). Level of education, income, crop yield, size of farm, social participation, level of participation in extension classes may well explain for 53% (R^2=0.534) changes in farmer's knowledge regarding innovation management (Table 4).

Table 1: Farmer's knowledge regarding innovation management in farming cooperatives

Level of Knowledge	f	%	Cum%
Very Low	12	11.4	11.4
Low	14	13.3	24.8
Moderate	54	41.4	76.2
High	13	12.4	88.6
Very High	12	11.4	100
Sum	105	100	

Table 2: Farmer's knowledge regarding each item of innovation management

Factors	Number of items	Mean*	sd
Innovation designing	9	2.653	1.08
Innovation planning	11	2.091	1.01
Innovation organizing	10	2.761	0.93
Innovation diffusion	8	3.112	1.04

*: 1=very low, 2=low, 3=moderate, 4=high, 5= very high

Table 3: Correlation between selected variables

Variable	r	p
Accessing to Communication channels	0.712	0.000***
Crop yield	0.632	0.000***
Size of farm	0.411	0.000***
Social participation	0.649	0.000***
Income	0.517	0.000***
Participation in extension	0.340	0.000***
Level of education	0.381	0.000***

Note. *: p<0.05; **: p<0.01; ***: p<0.001

Table 4: Liner regression results for predicting changes in knowledge of farmers

Variables	B	SE B	Beta	T	Tsig
Crop yield	0.423	0.452	0.543	3.543	0.000
Size of farm	0.165	0.354	0.443	2.432	0.000
Social participation	0.622	0.454	0.214	3.343	0.000
Income	0.411	0.543	0.812	3.981	0.000
Extension classes	0.391	0.344	0.091	2.813	0.000
Level of education	0.409	0.432	0.410	4.877	0.000
Accessing to Communication channels	0.232	0.612	0.523	3.213	0.000
Constant	4.651	1.005	-	4.678	0.000
F= 12.340	Signif F= 0.000		R^2=0.534		

This relationship is described in the following formula**:**

$Y = A + b1X1 + b2X2 + ...$

$Y = 4.651 + 0.423x_1 + 0.165\ x_2 + 0.622x_3 + 0.411\ x_4 + 0.391x_5 + 0.409x_6 + 0.232x_7$

Discussion

Innovation is associated with the introduction of new activities on the market (Kwamena, 2008). Innovation management is the economic implementation of new ideas and discoveries, and the implementation of an innovation culture in an organization, to promote and make possible the development of new ideas and business opportunities. Innovation management consists of innovation strategy, culture, idea management and implementation of innovation processes (Riederer et al, 2005, p. 4).

According to results the people with high education level, accessing to communication channels, income, training and social status had higher knowledge to innovation management in their business. The some of this finding was supported by Bylin et al (2004), Fulton et al (2003) Kwamena (2008), Quinn (1999), Riederer et al (2005) and Coash et al (2003), Reeve and Black (1998) .

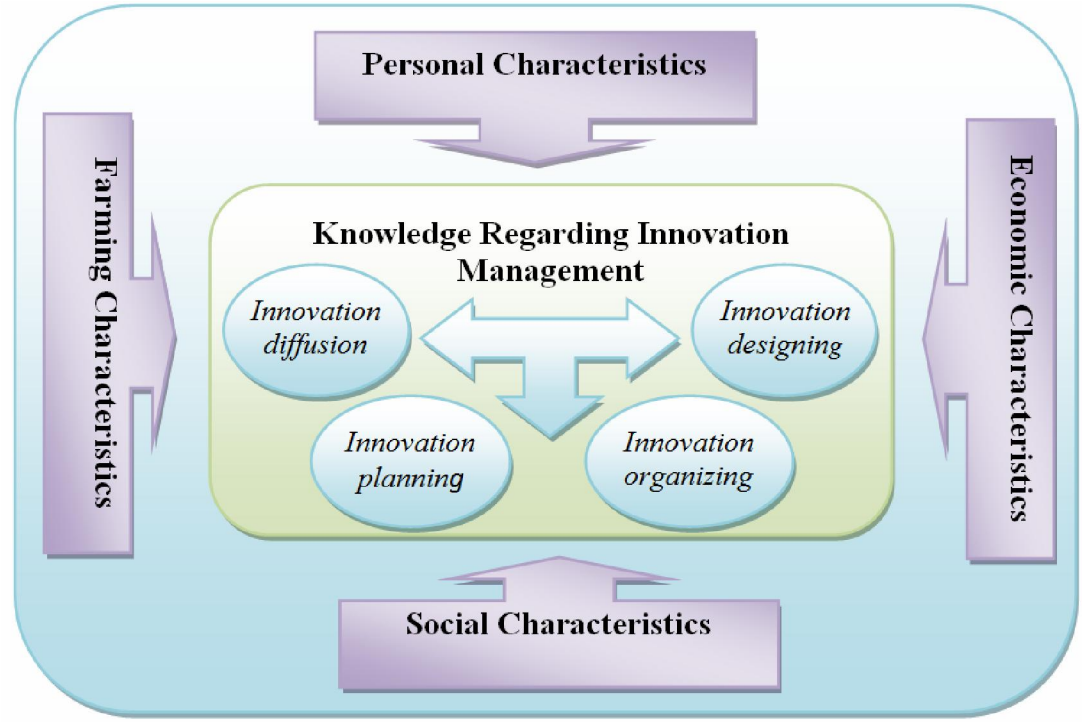

Figure 2: Filed Framework of Research

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Calculate effects of synergism and antagonism of nutrient elements: nitrogen, phosphorus, potassium and sodium in maize

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Abstract: Research projects in three consecutive years in 1999-2000 &2000-2001 and 2001-2002 years. Research Station - Research Azad University of Ahvaz were performed every three years in corn research using factorial experiment with a randomized complete block design with base 4 replications and two water stress factor with four levels as the first factor (**I_0** : Full irrigation point of FC, control, without water stress, **I_1**: 75% of the amount of irrigation treatments I_0, mild stress, **I_2**: 50% of the amount of irrigation treatments I_0, severe stress, I_3: 25% of the amount of irrigation treatment I_0, very severe stress and point of PWP) , period of growth with three levels as the second factor (V_1: vegetative period (until the emergence of the first deployment of plant double ring) V_2: reproductive period, V_3: the grain filling period in 3 years (1999-2000 &2000-2001 and 2001-2002) (Research Station, Islamic Azad University of Ahvaz 3 km south of Ahvaz city was designed and executed. Fertilizer amounts given in the first and second year experiment (1999-2000 &2000-2001) the same (N_{180} P_{70} K_0) was the third year of experiment (2001-2002) 20 percent of the amount of nitrogen and phosphorus fertilizers (N_{216} P_{84}) and the amount of 50 kg ha potassium fertilizer (K_2 O) to determine whether increased nutrient concentrations in the environment of plant roots in the same levels of water stress, changes in the process of accumulation of these elements in plant leaves, or not? Test results gathering process cluster to compare nutrient nitrogen, phosphorus, potassium and sodium in Different levels of water stress showed that the process of absorption and accumulation of nitrogen and phosphorus, two elements as well as potassium and sodium exclusively with each other at 1% level were similar. And because this was similar to that imposed different levels of water stress accumulation amount of both nitrogen and phosphorus element in the plant decreased, but the same amount of respect, two elements of K^+ Plant showed an increasing trend Regression analysis of variance in nutrient interaction at different levels of water stress, nutrient interaction with nitrogen phosphorus level of 5%, sodium potassium, nitrogen and potassium at 1% level significant effects on the interaction of elements and showed sodium diet with phosphorus, potassium and sodium phosphate with nitrogen did not provide significant effects. P interaction with N elements with correlation coefficient, linear regression fit showed that with increasing accumulation of nitrogen, phosphorus accumulation also increased with exercise and stress levels decrease Nitrogen accumulation was. Phosphorus accumulation process also presented a significant decrease. fit linear regression interaction of sodium with potassium correlation coefficient showed that whatever amount was increased accumulation of potassium, sodium accumulation process of adjustment and provide significant levels Severe water stress that was greater accumulation of K, the process of absorption and accumulation of sodium than the control treatments (water stress) and mild stress (treatments) can be reduced. Increasing the nitrogen element, additive effect on the accumulation process with correlation coefficient K said that the effects on the control treatment (no water stress) was more evident at different levels of water stress by reducing nitrogen absorption, accumulation of ions to a very moderate state control part of his indicate that if the absorption of nitrogen in different treatments of water stress was not reduced, ion accumulation in the treatments than values obtained was estimated.

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Key words: synergism & antagonism, nutrient elements, maize

1. Interoduction

Process of absorption and accumulation of nitrogen in corn because of the importance of this element in the growth and metabolism also play an important parameter for production and yield stability in the early stages of growth and value will be severely nitrogen during leaf 75 percent total nitrogen seems, rapid nitrogen accumulation process maturity seeds continue until the beginning of grain, 88 percent of plant N uptake, and only the remaining 12 percent is absorbed in the grain filling stage, the median between 25 between 75 days after green absorption is 65 percent nitrogen(1,18). When grain filling, nitrogen in leaf and stem and cob seed transmission 25 countries worldwide, announced that one of the important effects of drought which reduced the grain yield is the lack of absorption of nitrogen fertilizers is a significant limitation to the actual corn yield potential in these countries has created with the study of different sources, to determine the frequency of occurrence of drought and its effect on nitrogen uptake Research Station, plant response to nitrogen inputs

under different moisture regimes evaluation will provide is(5):

$$y = b_0 b_1 N + b_2 N^2 + b_3 D + b_4 D^2 + b_5 ND$$

In this formula, expected product of corn to nitrogen fertilizer application rate and a drought index number (D) the relevant coefficients are constant(5).

Process of absorption and accumulation of phosphorus in the plants need different periods, is different, 75-25 days after emergence, nearly 55 percent of phosphorus uptake of corn is needed, this nutrient during the whole growth period, along with stored dry material is done, phosphorus during formation and filling Seeds, transmitted to the body and 75 percent of the seeds will be stored between canopy and the emergence of flower seeds to reach half of the phosphorus is needed to save the plant and finish reports Taylor (1992), Gomez and Bltrans (1992), Prmachandra and colleagues (1993) confirmed the following points are:

1 - Water stress increases the absolute amount of nitrogen, phosphorus, and decreased amounts of different effects on the absorption process are K.

2 - Adult corn water stress, accumulation of phosphorus, nitrogen, magnesium and potassium were 40, 50, 60, 65 and 91 percent planted plants without water stress, decreased.

3 - More water stress than nitrogen sorption permanent wilting point affected by development.

4 - Water stress on the lower left of Ca absorption, and thus ratios Ca / p and Ca / k is high.

5 - Water stress activity of older roots can stop and just the tip of roots to absorb nutrients do that bivalent cautions to a greater capacity to absorb, anion absorption is also limited.

6 - In corn, water stress decreased absorption of phosphorus, and has a direct linear relationship (17).

For plants such as corn and grain and fine grain that have short growing season, phosphorus solubility in water is very important, but for plants with a long growing season pasture plants such as these have less importance because during their long growing season gradually Solubility P2O5 occurs, increase in soil phosphorus solubility, increased corn production will be substantially Potassium absorption process in the early stages of growth, compared with dry matter accumulation is very high, which is why if potash deficiency, young corn plants show it. At the beginning of milky stage of maximum absorption of potassium in plant and at maturity occurs when grain, 2.3 potash in the leaves and 3.1 of its seeds have been saved. before the formation of potassium absorption aggregate one hundred percent and completely performed in compared with the elements nitrogen and phosphorus, potash plant gathering process, 30 days earlier reaches its maximum, and thus absorb potassium plant several weeks before the stop To be absorbed approximately potassium uptake in plant nitrogen has been reported When the ions K + sufficient and accessible, the condition that the amount of ions of potassium and the percentage of moisture is the result of corn absorbed K + completely done and the substitution K + by Na very minor and insignificant, the course in the absence of K and moisture content low, ions Na + and its absorption more was that salinity in the corn shows, comparing the size of ion hydrated 0.331 = K + and 0.358 = Na + nanometers, absorb ions of K + moisture adequate and optimal concentration of potassium, is more evident(10,17).

2. Material and Method

Research projects in three consecutive years in 1999-2000 &2000-2001 and 2001-2002 years. Research Station - Research Azad University of Ahvaz were performed every three years and as below soil samples were taken:

- **Before planting and Sprinkle fertilizer**
- **After harvest and collect the full product**

Samples from two 30-0 and 60-30 cm depth to the number 5 spot for each depth was performed separately for physical and chemical characteristics of soil, nutrients and determine the percentage Soil organic material was sent to the laboratory, the aim of measuring soil nutrients after the harvest is that the discharge process or absorb nutrients in the field will be investigated and evaluated (Table 1).

Table 1: Results of soil analysis

soil	Deep (cm)	EC	Organic matter (%)	PH	Nitrogen (ppm)
Silty	0-15	6.5	0.6	7.7	635
Silty	15-30	6.6	0.3	7.6	648
Clay loam	30-60	5.7	-	7.3	211

Curve to determine the characteristics of soil moisture, samples 30-0 and 60-30 cm depth by the drill samples were taken, these samples by method of pressure plates under pressure 0.1, 0.3, 1, 3, 5 and 15 were modified and the soil moisture curve in a field oriented coordinate system based on volumetric soil water potential and percent moisture were plotted (Figure 1) prepared purpose of this curve to determine the amount of water

stored in Soil water potential in each soil, how soil and water use in determining soil moisture Tension meter or vice versa, to convert moisture to soil water potential was used.

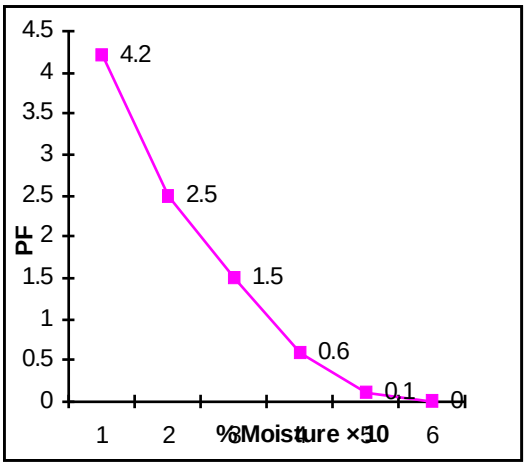

Figure 1: Curve of soil moisture field

Research projects in the form of factorial experiment with the basic design with two randomized complete block with four replications factor and mathematical model was performed following a three-year basis;

$$X_{ijk} = \mu + \delta_i + \delta_j + \delta_k + \delta_{jk} + \varepsilon_{ijk}$$

In this model, each view X_{iik} value, the average population, the effect of first factor, the effect of the second factor, the effect of blocks, Interaction first and second factor and the effect of experimental error is. Because the use of a factorial Experiment to prevent mixing of (soil × irrigation) and complete separation from the plot, to prevent water penetration to the adjacent plots and also the importance of the same first factor and the second is (Table 2).

Table 2: Review of different treatments tested

Main plot: Drought stress Levels	**Sub-plots: Different growth phases**
I_0 : Full irrigation point of FC, control, without water stress	**S_0**: growing phase, the establishment of the plant stem to the emergence
I_1 : 75% of the amount of irrigation treatments I_0, mild stress	**S_1**: natal phase: to stem the rise of coffee being resilient and end silk pollination
I_2 : 50% of the amount of irrigation treatments I_0, severe stress	**S_2**: grain filling phase: the end of pollen grain maturity and the emergence of black layer
I_3 : 25% of the amount of irrigation treatment I_0, very severe stress and point of PWP	-

For conducting water pressure care we used pressure layers, Field capacity points (FC), permanent wilting point (PWP) that equals 24.6 and 14.7 respectively, then we measured the special apparent weight of soil by using cylindrical, volume meter equals to 1.3 pa g/ cm^3. These three parameter: FC, PWP, Pa are considered as constant during experiment. Soil moisture means every other day calculated in sampling by the following formula:

Weight moisture percentage:

$$\%\theta_V = \frac{W_1 - W_2}{V} \times 100$$

Volume moisture percentage:

$$\%\theta_M = \frac{W_1 - W_2}{V_2} \times 100$$

W_1: the weight of moist soil.
W_2: the weight of this sample soil after soil dried.

Since the volume of sampling drill cylinder (V) is stable so calculate soil moisture volume percentage and using calculated parameters, calculate the amount of water entered into each part of land (each small part of land is enclosed so that water could not flow out) by using this formula:

$$V = \frac{\theta_V . A D_S}{E} = \frac{\theta_m . P_a A D_S}{E} = \frac{(Fc - pwp) p_a . A D_S}{E}$$

V: is equal to amount of water necessary for each irrigation

$\theta v(Cm^3)$: equals to volume moisture percentage

θm : equals to weight moisture percentage

P_a: equals to apparent weight of soil. (G/cm3)
Fc: Field capacity points
PWP: permanent wilting point.
A: equals to experimental plate level (cm^2)
E: equals to irrigation efficiency
D_s: equals to depth of root penetration (used tranche excavation) by installing parshal folum and meter we measured the amount of water entered into each part of land.

Sampled each plate based on 1m length ($100 \times 75 cm^2$) one time in fourteen days. In each sample we planted 4-5 corn plant in plastic bag so that we could analyze the nutrient elements in plants. Installing Parshal Flume and also taking water meter, the amount of water input to each plot and control were applied.

Methods to estimate nutrient nitrogen, phosphorus, potassium and sodium

Determine the nutrient once every 14 days from each plot based on a linear meters (75×100 Cm^2) were sampled, 5-4 plants in each sample after placing in a plastic bag immediately to the laboratory and analyzed to determine the nutrient

was sent, that some of these samples to determine nutrient as follows were used:

1 - the principle; experiment, part of the plant that the best indication of the status element accumulation in plants Offering to give; high section, middle and lower plant samples weighing 2 g were prepared. Height Sample preparation (A = 70, B = 70-140, C> 140 Cm) from top floor was considered.
2 - Based on the recommendations of the researchers of Pennsylvania State University and Illinois, the best tissue samples to measure changes in plant nitrogen, plant and upper measuring phosphorus and potassium, lower part of the plant, which is in addition to implementing these recommendations, the average of The samples were estimated.
3 - Measurement and estimation of plant N Kjeldal method and apparatus by distillation using the following formula:

$$= (T - S) \times N \times \frac{14}{1000} \times \frac{100}{Dryweight(g)} N_1$$

Amount of nitrogen in the sample based on percentage Normality sulfuric acid standard solution. N: sulfuric acid used for making Titration plant samples. S: sulfuric acid used for making Titration control.
4 - Measurement and estimation of ammonium Mulibdate by calorimetric method, using 2 grams of plant samples were dried, and then the samples were mixed with magnesium nitrate and postoperative get ashes , the ash in acid solution, and then smooth Finally phosphate was Calorimetric method were measured.
5 - Measurement of potassium and sodium in plants: the amount of 2 g of plant sample was beaten and practice get ashes dry heat than 480 ° C was performed after the ash in acid solution with a photometer methods to estimate the size of two elements were measured. To determine the number of sodium Samples from the device 2-5 Data analysis method and type of computer software Plan for statistical analysis of variance, raw data, a factorial experiment design based Randomized complete block with 4 replications that comparison data for analysis by a Duncan multiple range test was used. To perform analysis of variance and regression to determine the relationship between different variables, such as nutrient interactions, comparing the process of accumulation, the process of absorbing nitrogen and chlorophyll content, etc., MTB and software for the analysis of plant growth and seed MTB and SAS software and charts by 2000 Excel software were drawn. Atom - a spectral photometer was used.

Figure 2: View from the farm

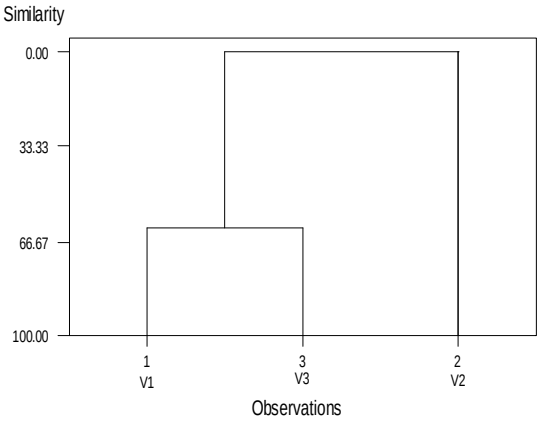

Figure 3: Comparison of the effect of drought stress accumulation process of nutrient nitrogen, phosphorus, potassium and sodium.

3. Result

Test results gathering process cluster to compare nutrient nitrogen, phosphorus, potassium and sodium different levels of water stress showed that the process of absorption and accumulation of nitrogen and phosphorus, two elements as well as potassium and sodium, Exclusively with each other at 1% level were similar. And because this was similar to that imposed various levels of water stress accumulation amount of both nitrogen and

phosphorus element in plants, but decreased the amount of respect, two elements of K^+ Plant showed an increasing trend (Table 3 and Figure 3).

Comparison of uptake and accumulation process of nutrient nitrogen, phosphorus, potassium and sodium in different periods showed that the growth process of absorption and accumulation period of growth (vegetative phase until the formation of ring Double) and (grain filling phase) more than similar treatments (after double-ring formation and Reproductive period) were in the process of accumulation and growth period was much lower than in the test cluster results in a classification system were replaced. (Figure 4 Table 4).

Table 3: Results of tests for comparison of cluster process nutrient uptake at different levels of water tension

Grand centered	Cluster 3	Cluster 2	Cluster 1	Variable
1.7375	1.47	1.78	1.85	***N (%)***
0.99	0.58	1.07	1.55	***P(ppm)***
2.1925	2.45	2.36	1.98	***K (%)***
0.5525	1.04	0.8	0.185	***Na (%)***

Center	Cluster	Between	Distance
	Cluster 3	*Cluster 2*	***Cluster 1***
1.1946	0.7313	0.00	***Cluster 1***
0.6340	0.00	0.7313	***Cluster 2***
0.000	0.634	1.1946	***Cluster 3***

Distance Level	Similarity Level	Number of cluster	Step
1.491	65.47	3	1
2.344	45.72	2	2
4.319	0.00	1	3

Comparison of uptake and accumulation trends in nutrient and water stress interaction term growth by cluster test showed that the growth period and separately at different levels of water stress were very similar presentation and classification of this test were together (Table 4, Figure 5).

Table 4: Cluster test results to compare nutrient uptake process in different periods of plant growth

Grand centered	Cluster 2	Cluster 1	Variable
0.0000	1.0095	-0.5047	***N (%)***
-0.000	1.1536	-0.5768	***P(ppm)***
0.000	1.000	-0.5000	***K (%)***
0.000	1.1524	-0.5762	***Na (%)***

Center	Cluster	Between	Distance
	Cluster 2	*Cluster 1*	
	3.2443	0.000	*Cluster 1*
	0.000	3.2443	*Cluster 2*

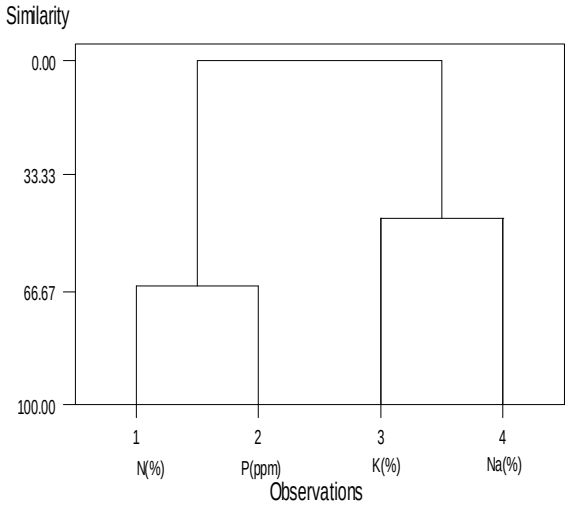

Figure 4: Comparing the assembly process nutrients nitrogen, phosphorus, potassium and sodium in different periods of growth.

Table 5: cluster test results to compare nutrient uptake process in the interaction of water stress and plant growth periods

Distance Level	Similarity Level	Number of cluster	Step
1.402	62.0	2	1
3.69	0.00	1	2
Distance Level	**Similarity Level**	**Number of cluster**	**Step**
0.231	94.89	11	1
0.380	91.59	10	2
0.43	90.49	9	3
0.600	86.72	8	4
0.841	81.39	7	5
1.248	72.38	6	6
1.455	67.80	5	7
1.998	55.77	4	8
2.623	41.94	3	9
3.042	32.65	2	10
4.518	0.00	1	11

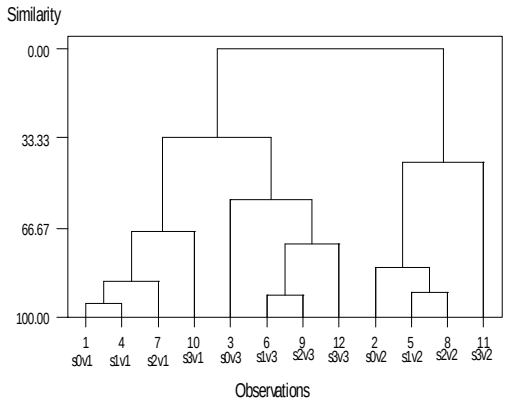

Figure 5: Comparison of nutrient accumulation process in the interaction of water stress and plant growth periods.

Nutrient interactions

With regression analysis of variance and presented as linear equations between nutrient, nutrient interaction on the process of gathering them at different levels of water stress and growth periods separately evaluated The following results were obtained:

1 - Regression analysis of variance in nutrient interaction at different levels of water stress, nutrient interaction with nitrogen phosphorus level of 5%, sodium potassium, nitrogen and potassium at 1% level significant effects on the interaction of elements and showed sodium diet with phosphorus, potassium and sodium phosphate with nitrogen did not provide significant effects. (Table 6)

2- P interaction element with the correlation coefficient with nitrogen, fitted linear regression showed that increased accumulation of nitrogen, phosphorus accumulation also increased by applying different levels of stress decrease Nitrogen accumulation was. Phosphorus accumulation process also offered a significant decrease (Figure 6).

3 - Fit linear regression interaction of sodium with potassium correlation coefficient showed that whatever amount was increased accumulation of potassium, sodium accumulation process of adjustment and provide significant levels Severe water stress that was greater accumulation of K, the process of absorption and accumulation of sodium than the control treatments (water stress) and mild stress (treatments) can be reduced. (Figure 6)

4 - Increase the element nitrogen, additive effect on the accumulation process with correlation coefficient K^+ said that the effects on the control treatment (no water stress) was more evident at different levels of water stress by reducing nitrogen absorption, accumulation of ions to a very moderate state control part of his indicate that if the absorption of nitrogen in different treatments was not reduced water stress, ion accumulation in the treatments than values obtained were estimated. (Figure 6) Regression analysis of variance in nutrient interaction in the different periods of growth, nutrient interaction with nitrogen phosphorus and potassium, with nitrogen, respectively 5% and 1% level, significant effects on each other showed and nutrient interactions with sodium potassium, phosphorus, sodium, potassium and sodium phosphate with nitrogen did not provide significant effects (Table 6).

The following results can be investing:

1 - the process of accumulation of nitrogen in the vegetative period of treatment, the highest accumulation of this element in plants showed that Periods of growth and accumulation, adjustment was significant, with increased accumulation of nitrogen in each phase of growth, P accumulation time significantly and can be significantly increased with decreasing nitrogen accumulation Phases of growth, and phosphorus accumulation in the two-phase growth was reduced, in this regard, a go to the incidence of positive correlation did. (Figure 7).

2 - Effect of N accumulation onion accumulation in different periods of plant growth from the accumulation of phosphorus (Part 1) was much higher than the correlation with N accumulation process in each period, the accumulation of potassium

To be significant indicated that the three periods of growth phase and the process of accumulation of both elements N and K in terms of value, had a high correlation (Figure 7).

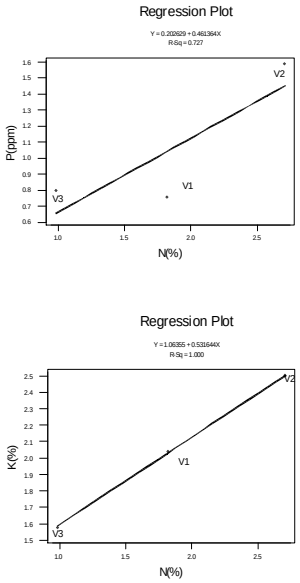

Figure 6: Fitting linear regression nutrient interaction at different levels of water stress
A) Nitrogen & phosphorus
B) Sodium & potassium
C) Potassium & nitrogen

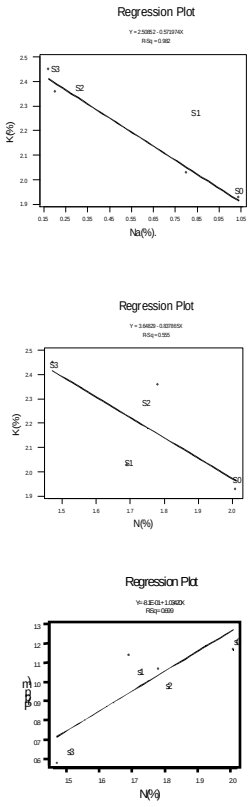

Figure 7: Fitting linear regression nutrient interaction in the different periods of growth

A) Nitrogen & phosphorus
B) Potassium & nitrogen

4- Discussion

Process of accumulation of nitrogen and phosphorus and potassium and sodium levels of water stress was also very similar process so that with increasing water stress, nitrogen and phosphorus accumulation in plants, but reduced accumulation of potassium and sodium in the plant increased (1, 17, and 5).

the process of accumulation of nitrogen, phosphorus and potash, especially nitrogen and potassium in corn also an increasing trend is similar, but Lopez (1992) proclaims that under water stress due to decreased absorption of nitrogen and phosphorus during the similarity of the process plant life process of change increased accumulation of potassium and sodium in a process similar to the levels of water stress were both single capacity of these elements under water stress were more absorbed in this connection about 50 times the absorption of potassium sodium adsorption was one of the reasons that attract little sodium presence of ions in the soil was tested certainly if the amount of potassium in the soil element was lower absorption of Sodium showed further increase(8).

Process of nutrient accumulation in different growth stages was different, the highest element in the process of gathering all four periods of plant growth was the highest growth rate was achieved, but the periods of growth and development was done in less and less because of this growth, less need elements had accumulation of N, phosphorus, potassium and sodium showed less (24).

The absorption and accumulation of nitrogen element in the plant increased accumulation of phosphorus and plant significant increase was in other words the process of absorption and accumulation of the element nitrogen phosphorus and potassium accumulation increased considerably increased Anthony (1998) proclaims that increased plant growth and nitrogen element required for the elements phosphorus and potash in order to increase the nitrogen element technique parameters such as root stages, resistance to water shortages, spread of LAI, etc. as necessary should that be due to increased water stress conditions reduce accumulation of potassium ions and nitrogen interaction of nitrogen and potassium, a negative trend shows that for increasing plant resistance to water stress and leaf area also decreased to prevent waste of water by sweating on different behavioral stress conditions takes (2000 cm) so impaired absorption and accumulation of ions such as nitrogen and other increased uptake increased potassium uptake is reduced in several reports by Mashner (1985) stated that plants, if any negative effect on ion absorption capacity of single-caution of other ions such as affects(1,10).

Table 6: Analysis of variance regression nutrient interaction at different levels of water stress

Mean Squared						df	Resource changes
Na & N	*K & P*	*Na & P*	*K & N*	*Na & K*	^{+}P & *N*		
$0.28^{n.s}$	$0.12^{n.s}$	$0.39^{n.s}$	0.38^{**}	0.56^{**}	0.21^{*}	1	**Regression**
0.14	0.036	0.086	0.02	0.0006	0.11	2	**Error**
50.4	61.7	69.7	55.5	97.6	69.9	-	R^2(%)
Na=2.96-1.38 N	**K=2.9-0.7P**	**Na=1.85-1.32 P**	**K=3.65-0.89N**	**Na=2.51-0.57 K**	**P=-0.8+1.03N**		**Linear regression equation**

*Each element being the first letter abbreviation represents the dependent variable (y) and the second letter independent variable (x) is.

Table 7: Analysis of variance regression nutrient interaction in the different periods of growth

Mean Squared						df	Resource changes
Na & N	*K & P*	*Na & P*	*K & N*	*Na & K*	^{+}P & *N*		
$0.49^{n.s}$	$3^{n.s}$	$0.006^{n.s}$	0.42^{**}	0.48^{ns}	0.38^{*}	1	**Regression**
0.2	0.12	0.003	0.0001	0.21	0.019	2	**Error**
70.8	71.2	58.3	100	69.3	72.2	-	R^2(%)
Na=-6.4+0.57N	**K=1.16+0.8P**	**Na=-1.01+1.2P**	**K=1.06+0.532 N**	**Na=-1.76+1.07K**	**P=0.203+0.4N**		**Linear regression equation**

*Each element being the first letter abbreviation represents the dependent variable (y) and the second letter independent variable (x) is.

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Organochlorine pesticides (OCPs) in Breast milk in Hong Kong-Review

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Abstract: Organochlorine pesticides (OCPs) contaminant in human breast milk research is an environmental indicator. Because, diet is a major factor that influences breast milk levels of persistent organic pollutants, with patterns in fish consumption playing a particularly significant role. In this paper review available data on levels of organochlorine pesticides (OCPs), polychlorinated dibenzodioxins (PCDDs) in breast milk of Hong Kong. After reviewing all available data demonstrated that organochlorine pesticides consumption in Hong Kong is decreasing according to time trend.
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Key Words: Organochlorine pesticides; Human milk; Hong Kong

1.Introduction

Organochlorine pesticides (OCPs) with their high persistence in the environment accumulate in fatty foods and human adipose tissues. Contamination of human milk by organochlorine and other related compounds has been reported throughout the world (GEMS, 1998). During the recent decade, investigations on POPs pollution in the Asian regions and found that relatively high residue levels of DDTs and HCHs exist in foodstuffs (Kannan et al., 1997), mussels (Monirith et al., 2003) and avian species (Kunisue et al., 2003) from some developing countries and these contaminants are possibly in use for public health purposes even now. Among Asian developing countries, concentrations of DDTs in human breast milk from Vietnam, mainland China, Cambodia, and Malaysia were relatively higher than those from other countries (Kunisue et al., 2004).

Human milk, at the top of the food chain, represents the major route of elimination of OCPs by lactating women (Rogan et al., 1986; Sim and Neil, 1992; IARC, 1991) concluded that there is insufficient evidence in humans but sufficient evidence in experimental animals to classify DDT as a possible carcinogenic to humans. However, body loads of DDT also raise concerns about potential effects on developing infants and children because DDT transfers across the placenta from mother to fetus and exposure continues through breastfeeding after birth (Shen et al., 2007).It is well known that they are very dangerous if ingested as an overdose but there is also biological evidence that chronic low-grade exposure to these chemicals, which are very easily absorbed into the body through the skin and lungs, may have adverse effects on mental health (Zhang et al., 2009).

The rapid socio-economic development during the past two decades in China, especially the coastal area and the Pearl River Delta has given rise to severe economic, environmental and health problems. In 1983 use of DDT in agriculture was banned. However, high residue concentrations for both two substances still existed in sediments, soils, foods and human organisms (Liu et al., 2003). In addition, technical DDTs remain in use in some parts of China for Malaria control and as additives in anti-fouling paints for fishing ships (Li et al., 2007).Organochlorine pesticide residues in animal tissues and other products are likely to occur from the accumulation of these lipophilic chemicals through the food chain and are closely associated with fats in the sample. In this paper review the sources of organochlorine in breast milk of Hong Kong and decrease level.

2.Usage of POP pesticides in China

Being one of the largest agricultural production country, China has been a major producer and consumer of pesticides, until its ban on production and agricultural use were enforced in 1983. China had been a significant producer and user of DDT since the 1950s. In Hong Kong Special-Administrative Region (SAR), DDT was banned from use on 31 December 1987, and currently can be traded only under permit (Wong et al., 2002).From 1979 to 1982, however, between 5032 and 5380 kg of DDT pesticide was imported into Hong Kong annually. Furthermore, there was a net gain of 736 tonnes of DDT between 1986 and 1988 in Hong Kong (Ip, 1990).DDT, HCH, toxaphene, HCB, chlordane, heptachlor, and mirex were produced in China. According to Stockholm Convention persistent organic pollutants (POPs) were banned in China and

Hong Kong as shown in Table 1

Table 1: Data on usage, ban and exemption from Stockholm Convention of POP pesticides in China and Hong Kong (Wong et al., 2002)

POPs pesticides	China				Hong Kong
	Starting of usage	Ban of usage	Production quantity (tones/year)	Request for Specific exemption	Ban of usage
Aldrin	Not used	1983 (2)	None	---	1988 (2)
Chlordane	1945	1999	160 (1998) 500[a]	Produced and used locally as a termiticide	1991 (2)
DDT	1950s	1983 (1)	4000-6000 3000-4000[a]	(building and dams) Use for control of mosquito and export to countries needed, based on prior	1988 (2)
Dieldrin	Not used	1983 (2)	None	informed consent procedures	1988 (2)
Endrin	Not used	----	None	(PIC)	Not
Heptachlor	1948	1982	1 (1969)	-----	registered
Hexachloro-benzene	1945	No information	1000-10000 3000-4000[a]	----- -----	Not registered
(HCB)				Produced and used locally as an intermediate in the	Not registered
Mirex	1958	Banned as pesticide	10-30[a]	production of other chlorinated substances.	
Toxaphene	1948	1982(2)	3000 (maximum Quantity in 1970s)	Produced and used locally as a termiticide with limited production and some local use.	1997 (2) 1984 (2)

Note: banned from agriculture (1); banned from all purposes (2).
[a] UNEP, 2005

Historically, there were about 60 POP pesticide-producing enterprises located in 18 provinces in China. The accumulated output of DDT, toxaphene, and HCB were 459,000, 20,660, and 79,278 MT, respectively, while the accumulated output of HCH was 141,366 MT during 1990-2003. In the 1980s, China set up the legal framework for controlling organochlorine pesticides, and the production and use of organochlorine pesticides for agriculture were banned. Presently, DDT and HCH can still be detected in air, water, sediment, field soil, grains, vegetables, fruits, meat, animals and human tissue in many areas. Suggest that government of China could keep the view in the pesticides problems and find the alternative ways to make sustainable environment. Pesticides were destroying the ecosystem and it brings the climate change issue in China.

The total amount of pesticides demand in 2008 was 298,200 tons nearly same as 2007. The pesticide varieties with more than 10,000 tons demand amount all over China will include dichlorvos, acetochlor, copper sulfate, glyphosate, trichlorfon, disosultap etc, 5,000 to 10,000 tons varieties include phoxim, carbendazim, monosultap, butachlor, atrazine, omethoate, triophanate-methyl, chlorpyrifos, 2,4-D butyl ester, dimethoate, acephate etc. In addition, the provinces with more than 20,000 tons of pesticide demand amount are Heilongjiang and Hunan, and the following provinces are ranging from 10,000 tons and 20,000 tons, like Guangdong, Yunan, Shandong, Henan, Anhui, Hubei, Hebei, Jiangsu, Liaoning, Guangxi, Fujian, Jiangxi, and Zhejiang.

Emission of Dioxin in Hong kong

The current and future polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) inventory for Hong Kong is lower (Table 2) than that for other national inventories per head of population, owing to a generally low level of combustion and industrial activity, but more significantly to the fact that Hong Kong has adopted a PCDD/F emission limit of 0.1 ng I-TEQ m^{-3} for all existing and proposed waste incineration facilities, in line with the best practice elsewhere.

Table 2: Estimated PCDD/F emissions to atmosphere from the HKSAR (1997-2007)

Sources	Activity 1997	Inventory (1997, g I-TEQ)	Activity (2007)	Inventry (2007 g I-TEQ)
Coal combustion	6.1 MT	0.4-2.0	5.6 MT	0.3-1.8
Municipal soil waste	116,508 t clinker	0.32	1000000	0.5
Clinical waste combustion	3,650 t	0.4-1.8	5,290t	0.2
Diesel	2515MKm	0.002-0.03	2515MKm	0.002-0.03

Estimate of daily dietary exposure to POPs

Human exposure to POPs through dietary intake was estimated based on measurement of the level of POPs contamination in various foods and information on daily food consumption pattern of the local population. Dietary exposure of Hong Kong residents to DDT, HCB, PCBs and dioxins/furans was estimated to be 29.3 ng, 1.11 ng, 8.31 ng and 0.91 pg-TEQ kg^{-1} bw d^{-1}, respectively. The major food groups contributing to POPs exposure were cereals, seafood's and dairy products.

The dietary exposure of the local population to DDT was comparable to the range reported in many European countries and the US (Herrera et al., 1996); higher than that in Australia (Miller et al., 2002) and New Zealand (http://www.pan-uk.org) but substantially lower (by 1–3 orders of magnitude) than that in Mainland China (Chen et al., 1993), Vietnam, India (Kannan et al., 1992) and Egypt (http://www.pan-uk.org) (Table 3).

Table 3: Comparison of dietary intake of POPs by the residents of Hong Kong and other urban locations.

Location	Dietary intake (ng kg^{-1} bw d^{-1})		Reference
	DDT	HCB	
Hong Kong, China	29.3	1.1	(EPD, 2005)
Australia	7	4.67	(Milleretal., 2002; EPD, 2005)
China, Mainland	341	-----	(Chen and Gao, 1993)
Egypt	13,700	-----	(http://www.pan-uk.org)
Findland	26	------	(http://www.pan-uk.org)
India	800	2.17	(Kannan et al., 1992)
New Zealand	3	------	(Buckland et al., 1998)
Slovakia	95.8	3.75	(Prachar et al., 1996)
Spain	20.3	17.2	(Herrera et al., 1996)
Switzerland	28.3	18.3	(Herrera et al., 1996)
Thailand	70	1.3	(Kannan et al., 1992)
Netherlands	16.7	3.33	(Brussard et al., 996;Freijer et al., 2001)
UK	50	-----	(FSA UK, 2003)
USA	26	0.5	(Herrera etal., 1996;USEPA, 2001)
Vietnam	320	1.7	(Malish, 1998)

The estimated dietary intake of HCB was found to be generally similar among residents of Hong Kong, other Asian countries/regions (Kannan et al., 1992), Australia (Miller et al., 2002; Kannan et al., 1995), the US (Herrera et al., 1996), the Netherlands (Brussard et al., 1996) and Slovakia (Prachar et al., 1996), but lower than the value reported in Spain or Switzerland (Herrera et al., 1996). Based on the few published data available for comparison, the level of local dietary PCBs exposure was found to be much lower than in other Asian countries (Kannan et al., 1992). The estimated dietary exposure of Hong Kong residents to dioxins/furans was generally comparable to the level reported in most European countries (Malisch, 1998), Canada and the U.S. (USEPA, 2001); slightly higher than the values recorded in Australia, New Zealand (Buckland et al., 1998) or the UK (FSA Uk, 2003), but appreciably lower than that found in Norway and Spain (Jimenez et al, 1996).

3.Organochlorine pesticides (OCPs) in Human milk

DDT levels in Hong Kong breast milk

The DDT levels in breast milk from Hong Kong indicate a marked decline in DDT human body load in Hong Kong in the past thirty years, assuming reasonable comparability of assay results in different previous surveys (Ip, 1983; 1989). Human breast milk is a convenient medium for monitoring levels of lipophilic organochlorine compounds such as DDT, first because of obvious concern over exposure to the suckling infant, but also because it is easier to obtain than, for example, adipose tissue (Sim and Neil, 1992).The most direct concern with toxic compounds in breast milk, of course, relates to potential exposure to the infant. While breast-feeding is usually considered to have overwhelming advantages (Newman, 1995), there has been concern that breastfeed infants in some areas could be exceeding recommended limits of various organochlorine compounds (Mitchell, 1997). Both *p,p′*-DDT and *p,p′*-DDE concentrations in breast milk in Hong Kong decreased over time. The ratio of *p,p′*-DDT to *p,p′*-DDE decreased from 0.38 in 1976 to 0.07 in 2002. This observation is consistent with a worldwide downward trend in DDT body load (Smith, 1999) and it was even suggested that the decline in average levels of DDT in breast milk in most countries was strongly correlated with the length of time since DDT restriction. However, the time trend in mainland China is not clear because there is only published data on the DDT body load in mainland China in recent years (Nakata et al., 2002; Kunisue et al., 2004).The level of DDT in human breast milk in Hong Kong was the second highest in the 1970s among 21 countries worldwide (Solomon and Weiss, 2002).The DDT produced and applied in south-east mainland China, where DDT had been extensively used, could also be an important source of exposure to Hong Kong residents. High levels of DDT in foodstuffs in Hong Kong have been reported (Ip, 1990).However, an apparent decreasing trend of organichlorine pesticides were observed in Hong Kong human milk 1980s further decrease was found in the (Hedley et al., 2010)(Figure 1).Lower levels of α-HCH and γ-HCH were also found compared with the 1985 level (Ip and Philips, 1989).

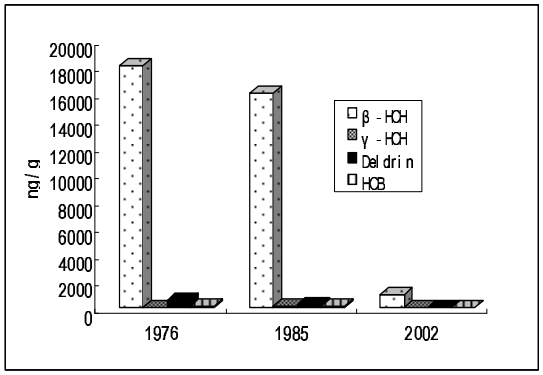

Figure 1.Time trend mean concentration (ng/g in fat) of OCP in breast milk in Hong Kong Review from (Ip, 1983, 1989; Hedley et al., 2010)

Comparison of OCP in breast milk Hong Kong with other countries

To understand the magnitude of contamination in human breast milk in Hong Kong, OCPs were compared with inside the China (Wong et al., 2002; Kunisue et al., 2004) further compared with other countries (Cok et al., 1997; Waliszewski et al.,2001; Hooper et al., 1997; Harris et al., 1999) as shown in (Table 4).

Table 4. Domestic and international comparison of organochlorine concentrations in human breast milk

Country	Year	DDTs	HCHs	Unit	Reference
China					
Hong Kong	1999	2900[b]	950[g]	ng/g lipid wt.	(Wong et al., 2002)
Hong Kong	2001	----	----	TEQ pg/g fat	(Soechitram et al., 2003)
Guangzhou	2000	3220-5980	700-2600	ng/g fat	(Wong et al., 2002)
Dalian	2004	2100[a]	1400[e]	ng/g lipid wt.	(Kunisue et al., 2004)
Shenyang	2004	870[a]	550[e]	ng/g lipid wt.	(Kunisue et al., 2004)
Developing and former socialist countries					
Turkey	1995-1996	2400[b]	480[e]	ng/g lipid wt.	(Cok et al., 1997)
Iran	1991	2000[b]	600[e]	ng/g lipid wt.	(Cok et al., 1997)
Mexico	1997-1998	4700[c]	60[e]	ng/g lipid wt.	(Waliszewski et al., 2001)
Kazakhstan	1994	2300[b]	1300[f]	ng/g lipid wt.	(Hooper et al., 1997)
Russia	1996	2000[b]	560[i]	ng/g lipid wt.	(Hansen et al., 1998)

Developed country					
Japan	1998	290[b]	210[g]	ng/g lipid wt.	(Konishi et al., 2001)
Sweden	1997	170[b]	---	ng/g lipid wt.	(Noren et al., 2000)
Germany	1995-1997	240[b]	40[g]	ng/g lipid wt.	(Schade and Heinzow, 1998)
Canada	1996	470[d]	23[e]	ng/g lipid wt.	(Newsome and Ryan, 1999)
UK	1997-1998	470[b]	100[h]	ng/g lipid wt.	(Harris et al., 1999)

[a] *p,p′*-DDE+*p,p′*-DDT+*p,p′*-DDD.
[b] *p,p′*-DDE+*p,p′*-DDT.
[c] *p,p′*-DDE+ *p,p′*-DDT+*p,p′*-DDD+ *o,p′*-DDT.
[d] *p,p′*-DDE+*p,p′*-DDT+*o,p′*-DDT.
[e] α-HCH+β-HCH+γ-HCH.
[f] α-HCH+β-HCH.
[g] β-HCH only.
[h] β-HCH+γ-HCH.
[i] Total.

Concentrations of DDTs and HCHs in human breast milk from Dalian were similar to those from Hong Kong and Guangzhou, while those from Shenyang were somewhat lower. This indicates that the residents living closer to the costa in China have been exposed to relatively high levels of DDTs and HCHs. However, further study in Hong Kong human milk found to be lower concentration of OCPs consumption. It demonstrated that uses of pesticides in Hong Kong were lower and peoples were following the permissible limit according to world health organization. The developing country like Mexico concentration of DDT was higher than Hong Kong, further total HCHs concentration in Kazakhstan also higher than Hong Kong. Hence, organochlorine pesticides consumption in Hong Kong is decreasing due to time trend.

4. Conclusion

We have review the available data/information of organochlorine pesticides contamination in breast milk of Hong Kong, China. In 1976 concentration of β-HCH was very higher than γ-HCH, deildrin and HCB. Further, monitored in 1985 and 2002 demonstrated that organochlorine pesticides concentration in Hong Kong breast milk found to be must lower than before. In view of our observations suggest that further investigation on human exposure in organochlorine pesticides are needed to elucidate future pollution trends and to assess infant health risk.

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Customer Complaints Management: Concepts and Applications

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ABSTRACT - In this paper, Customer Complaints Management (CCM) and its associated key challenges were studied as essentials for achieving customer retention and loyalty. Some models illustrating the process of CCM were also demonstrated and discussed. A complaint intensity framework is presented, in which the joint distribution of complaint intensity and outcome satisfaction scores are conceptualized in four resulting quadrants with each quadrant suggesting a different CCM strategy. In empowering CCM, suggestions are proposed and Return on Complaint Management (ROCM) is described as a performance indicator for complaint management profitability. Major findings indicate that effective complaints management requires a cultural change in organization's atmosphere, as well as a systematic approach; different levels should be considered in complaints management; employees participating in teams play an important role in succeeding the complaints handling processes; and CCM empowerment should include strategy, processes, and analysis.
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Keywords: Customer, Satisfaction, Complaints, Management, Handling, Empowerment.

1. Introduction

Complaints are a natural consequence of any service activity because "Mistakes are an unavoidable feature of all human endearment and thus also of service delivery" (Boshoff, 2007). Service recovery is the process of putting the situation right (Zemke & Schaaf, 2000) though it has been defined more widely and more proactively as the action of seeking out and dealing with failures in the delivery of service (Johnston, 2005). The term "complaint management" is used to include service recovery and involves the receipt, investigation, settlement and prevention of customer complaints, and recovery of the customer.

Firms normally consider consumer complaints of any kind to be indispensable indicators of unsatisfactory performance. Without consumers' feedback, they will be unaware of their problems and retain their customers (Crie & Ladwein, 2002). Lau and Ng (2001) found that dissatisfied consumers who complained had a higher level of repurchase intention than those who did not complain (Lau & Ng, 2001).

However, previous studies have also shown that many unsatisfied consumers prefer to change brands or suppliers and tell friends or families about their bad purchase experience than to voice their dissatisfaction to companies (Day & Ash, 2007). For these reasons, it is clearly evident that CCM needs serious attention.

Most organizations that face big challenges in customer complaints handling:

- Suffer from a lack of systematic approach to complaints handling.
- Do not recognize the importance of customer complaints on a strategic level.
- Are ill – equipped in terms of systems and processes for logging in complaints, processing them, etc.
- Are not proficient with measurement and particularly in non – financial areas such as customer satisfaction and complaints.
- Have adverse cultures and too much of "blame and reprimand" practices.
- Have not embraced the concept of quality management and its related concepts.

Customer satisfaction is not an absolute scenario, but very much depends on interaction, feedback, praise, and complaints. Complaints must be scrutinized in a constructive, positive and professional perspective:

- They are a way of receiving feedback from customers and therefore are necessary means for putting into action improvement plans.
- They are a tool for preventing complacency and harnessing internal competencies for optimizing products and services.
- They are a useful way of measuring performance and allocating resources improving the deficient areas of the business.
- They are a useful "mirror" for gauging internal performance against competition and the best in class organizations.
- They are a useful exercise in building relationship with the customer and understanding them better.

2. Complaining reactions

Consumers have various alternatives to express their dissatisfactions (Singh, 2000). Several typologies have been proposed to differentiate complainers from non – complainers (Crie & Ladwein, 2002). In general, four sets of actions can be summarized from the literature.

First, dissatisfied consumers can not take any action based on their bad/unfavorable buying experience. Doing nothing or not repurchasing a firm's product or services are legitimate responses to dissatisfaction (Mowen & Minor, 2008). Singh (2000) classifies consumers engaging in such behavior as "Passives" (Stauss, 2004).

Second, consumers can also take some form of private actions. Private actions refer to actions involving only people inside the consumer's group in informal ways. This may include changing the brand Supplier, ceasing to use the product or service, or warning family and friends. Private actions are more likely to be driven by "getting even and punitive aims" (Zeithaml, 2000). This group is classified as "Voicers" (Stauss, 2004).

Third, consumers can take some form of public actions. Public actions involve people and organizations outside the consumer's group in more formal ways. These may be seeking redress directly from the seller or manufacturer and taking legal action against the seller of manufacturer.

They may also be registering a complaint with the seller of manufacturer, a public consumer protection agency, or a private consumer organization (Day, 2007). Complainers may also create a new company to provide a better product or service (Day, 2001). The main purpose of these consumer complaints is to "recover economic loss by getting an exchange or a refund and rebuild self – image" (Davidow, 2007). This group is classified as "Irates" (Stauss, 2004).

Finally, consumers may take a variety of different private and public actions. Consumers may blame sellers and manufacturers for their unsatisfactory product or service. They may choose to boycott sellers and manufacturers by ceasing to use their products or services and spread negative information about their products or services. (Zeithaml, 2000). This last group is classified as "Activists" (Stauss & Seidel, 2004).

3. Models of CCM

Johnston (2001) proposed a model, based on an assumption that the prime purpose of designing and developing robust and effective CCM systems is to deliver empowered profits by increasing revenues and reducing costs (Figure 1) (Richins, 2003). As it is shown, complaint processes as the core of the model influences customer satisfaction, process improvement, and employee attitude.

In the following two more models of CCM, which have been used successfully in Boeing Aircraft and Tanker (Boeing A & T) and in the National Roads and Motorists' Association (NRMA), are demonstrated.

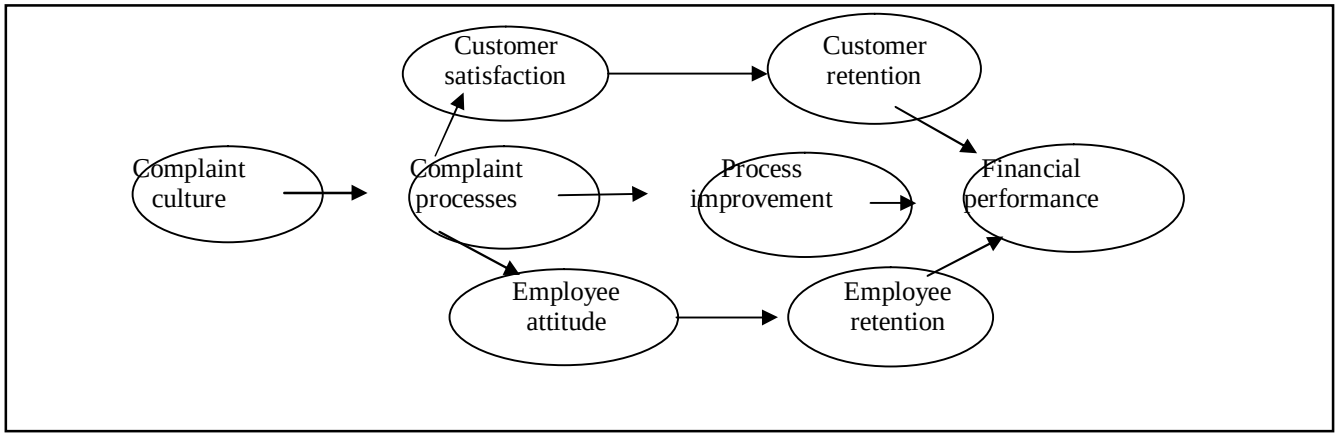

Figure 1. A conceptual model for the relationship between complaint culture (handling), customer satisfaction, and profitability (Johnston, 2005)

3.1 Boeing A & T

Winners of the Malcolm Baldrige National Quality Award, the Boeing Company Airlift and Tanker Program (A & T) designs, manufactures and supports aircraft for both passengers and cargo transportation. They serve four major markets and three major customer groups. Their primary customer is the US Air Force. Boeing A & T use different approaches in handling customer complaints. Amongst the key aspects of Boeing's complaints handling system is proactive management of customer contacts and complaint resolution through joint teams (Figure 2).

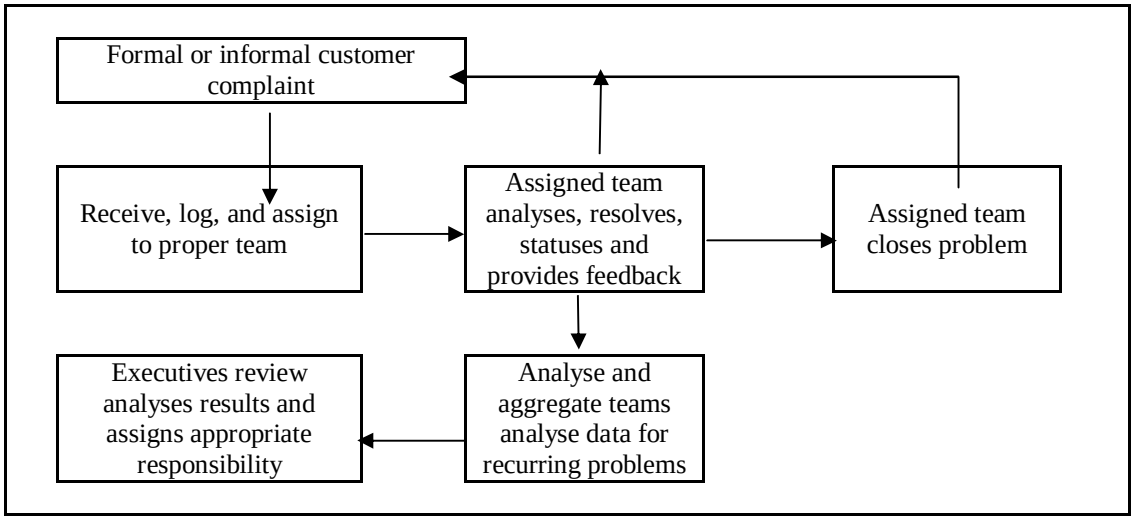

Figure 2. Complaint management model at Boeing A & T (Singh, 2008)

3.2 National Roads and Motorists Association (NRMA)

Winner of the Australian Quality Award in 1992, the NRMA was set up to provide services to road users and to promote the interests of motorists. Some of the services provided by NRMA include emergency road service, insurance, investment advice, finance, technical, legal, travel & touring amongst others. NRMA manages complaints through different principles and a three level model (Figure 3), which provides a comprehensive and systematic way to deal with complaints.

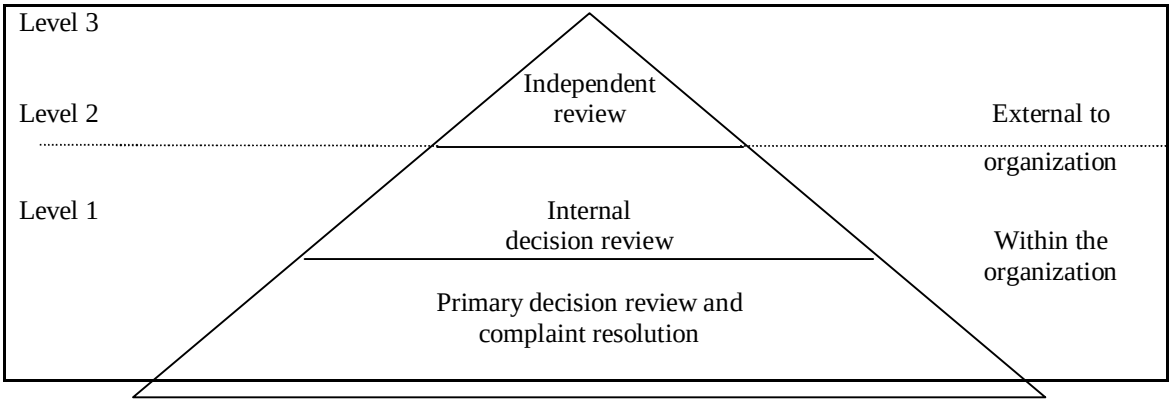

Figure 3. Complaint management model at NRMA (Singh, 2008)

According to the figure 3, it is concluded that a CCM system has different levels, namely internal and external, depending on the decision-making process, which in fact may be affected by organizational strategies.

4. Comprehensive CCM system

Although important research has been conducted around CCM system, most models are not comprehensive enough. Therefore, a model for CCM system that integrates practice – tested methodologies such as quality function deployment (QFD), problem solving and failure modes and effects analysis (FMEA)

was recently developed by Bosch and Enriquez (2005) (Figure 4) (Krapfel, 2005).
They also proposed three important indicators for measuring the system of CCM success as:

(1) time to respond to a customer complaint, from receiving it to giving an answer to the affected customer;
(2) percentage of closed cases out of complaints received; and
(3) evaluation of service level.

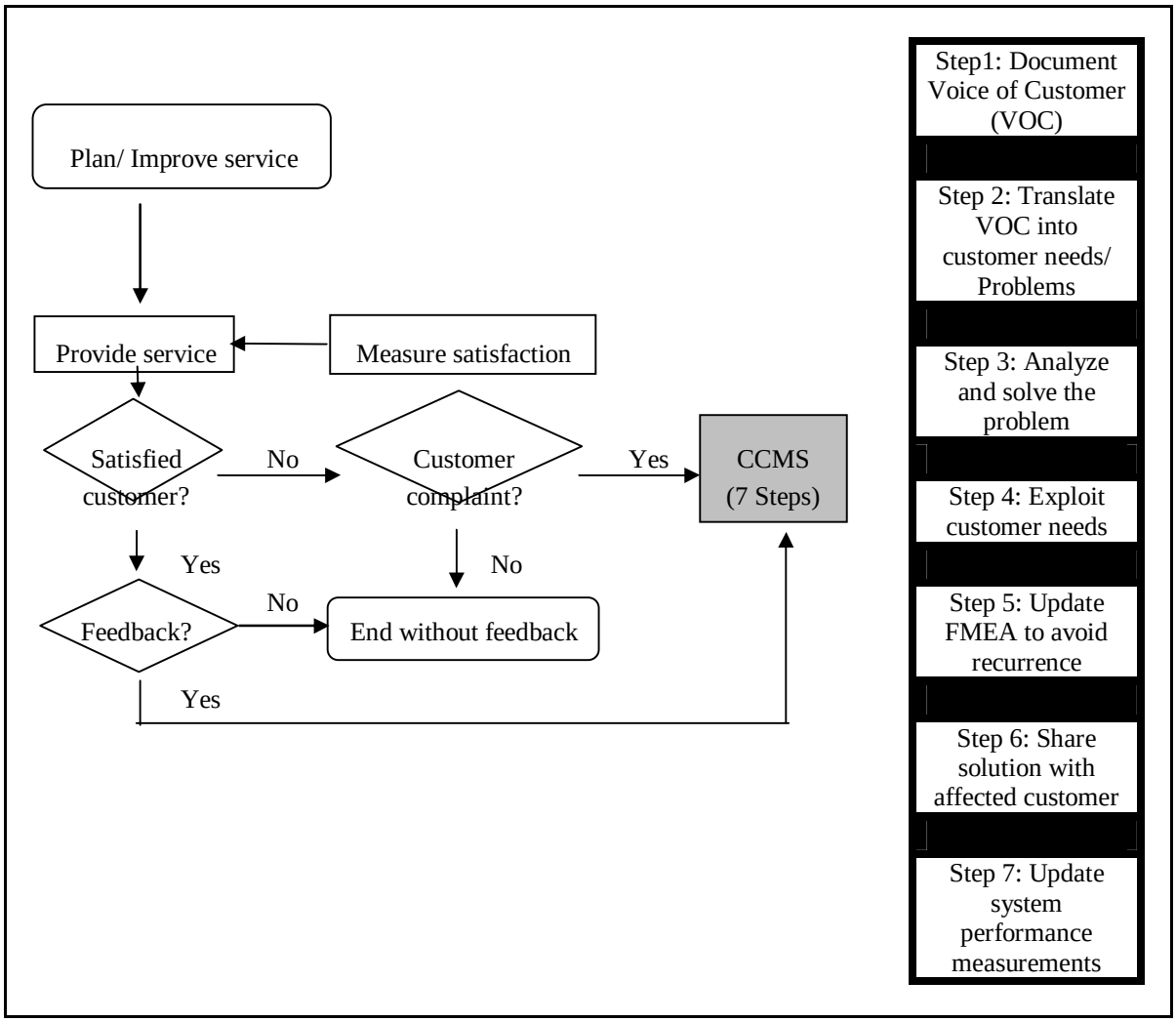

Figure 4. A comprehensive CCM system (Krapfel, 2005)

5. The complaint intensity outcome framework

The complaint intensity outcome framework is presented in figure 5. Points along the vertical axis indicate customers' mean attribute complaint intensity scores. The complaint intensity score for a given attribute is equal to the frequency of complaints for that attribute weighted by (i.e. multiplied by) the mean degree of importance which individuals who have complained about that attribute attached to such complaints.

As indicated in figure 5, the joint distribution of complaint intensity and outcome satisfaction scores is readily conceptualized in four resulting quadrants. Each quadrant suggests a different strategy.

The attributes found in quadrant I require primary attention owing to their high complaint intensity scores and low outcome satisfaction scores. Accordingly, an immediate – focus strategy is suggested.

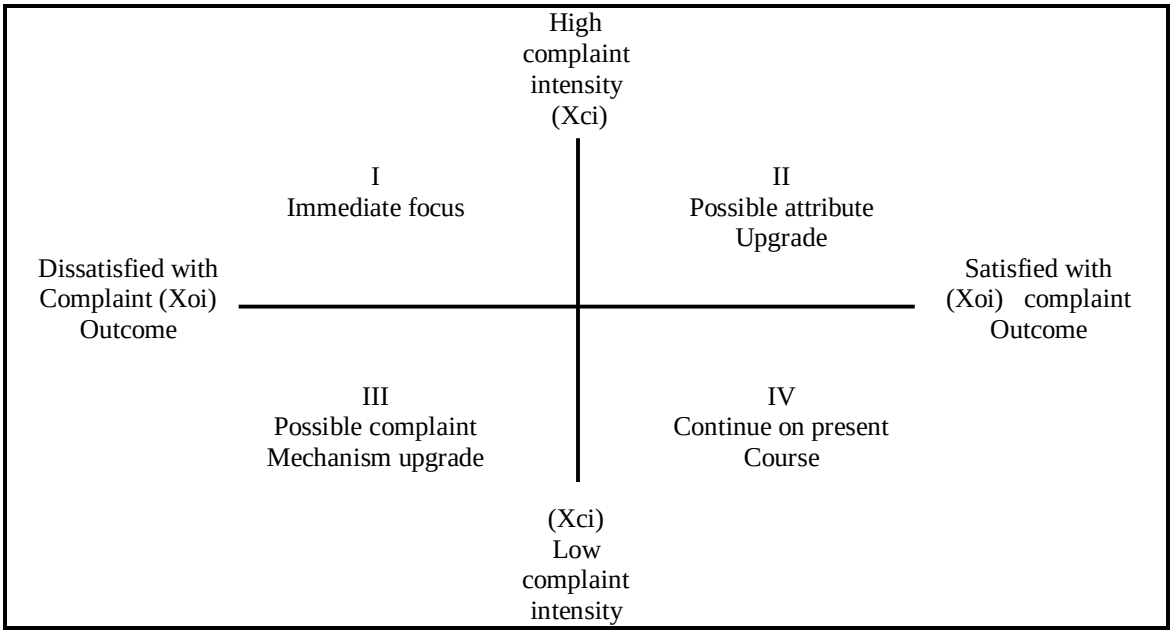

Figure 5. Complaint intensity outcome framework (Krishnan & Valle, 2007)

6. CCM empowerment

Empowerment is a powerful means of increasing customer satisfaction when resolving customer complaints. For successful empowerment application, however, it must be promoted within a framework which includes:

- encouraging service providers to take a positive and proactive approach to complaints;
- developing service providers' skills in handling complaints; being explicit about the level of authority employees have in complaint management;
- providing support and encouragement to employees in taking responsibility; and taking action to overcome the causes of complaints and generating ownership of the improvement opportunities that complaints bring.

7. Return on complaint management (RoCM) as a performance indicator for complaint management profitability

Complaint management profitability (CMP) represents the economic efficiency of the processes and instruments of complaint management systems. CMP is calculated by relating the invested capital to the profit of complaint management. The profit of complaint management is calculated by deducting its costs from its benefits. The invested capital equals the costs of CCM activities within period.

However, in order to calculate CMP, sufficient data are necessary. Furthermore, it has to be discussed which costs and benefits to include in this calculation, how to measure the costs, and how to express the benefits monetarily.

Regarding the costs of CCM, various types can be identified in the context of complaint management. These are described in the following (Davidow & Dacin, 2007):

(1) Personnel costs arise from human resources that are directly concerned with complaint management processes (e.g. staff of a complaint management department).

(2) Administration costs are generated by expenditures for, e. g. office space and office equipment.

(3) Communication costs are all costs that are associated with necessary communication processes to solve the customer's problem (e.g. phone costs or postage).

(4) Response costs are all costs that arise in the context of the problem solution. Here three types of response costs can be differentiated

Regarding the benefits of complaint management, four distinct types can be identified on the basis of literature analyses and expert interviews (Singh & Wilkes, 2006):

(1) The information benefit represents the value that is generated by using information from customer complaints to improve products, to enhance efficiency and to reduce failure costs.

(2) The attitude benefit comprehends the positive attitude changes of the customer due to achieved complaint satisfaction.

(3) The repurchase benefit arises when a complaining customer remains with a company instead of switching to a competitor.
(4) Communication benefits describe the oral effect of complaint management. They are generated when complaints are solved and satisfied customers are engaging in positive word – of – mouth, that is, recommending the company and by that supporting the acquisition of new customers.

To calculate CMP, it is necessary to have/maintain/keep operational the four types of benefits and to value them monetarily. The sum of the benefits minus the measured costs equals the profit of CCM. To calculate the return on complaint management (RoCM), (Figure 6), which is the key indicator for complaint management profitability; the profit of complaint management is set against the complaint management investments (costs).

$$\text{Return on complaint Management} = \frac{\text{Complaint management profit}}{\text{Investments in complaint management}}$$

Figure 6. Calculating the return on complaint management (Singh, 2008)

8. CCM and customer retention

The repurchase benefit of CCM is achieved when previously dissatisfied customers, who otherwise would have migrated, remain loyal to the company as a result of complaint management activities.

There are different approaches to calculate this effect. The following example is based on average data. The repurchase benefit is basically calculated in a way that the number of customers who remain loyal because of their experience with the CCM is determined. This number is then weighted with a customer's average profitability.
To be able to do this calculation, the following data are necessary:
- the total number of customers (customer base);
- the number of complainants;
- the share of convinced and satisfied complainants;
- their loyalty quota; and
- the percentage of complainants whose actual loyalty can be directly traced to complaint handling.

9. Conclusions

In this paper, some models and concepts of managing customer complaints were studied and important suggestions were proposed in order to empower the existing CCM system. It was found that managers deal with different challenges such as diverse cultural issues in managing CCM systems and employing quality management approaches in such systems. Culture is an important issue which could affect customer satisfaction, complaint processes and employee attitudes, all together resulting in organizational profitability. Based on the reviewed models, it was also emphasized that a CCM system may have different levels, both internal and external, in which different processes are analyze with respect to organizational strategies. A comprehensive system of CCM was also presented composed of various steps. Moreover, teamwork was addressed as a critical success factor in managing customer complaints. Besides the advantages of teamwork. It seems necessary to provide training to service providers in the skills, attitudes, and behaviors to deal positively and empathetically with difficult situations as a key step encourage confidence in empowering the staff. A well – trained and empowered member of staff can help transforming a dissatisfied customer into an advocate of the organization.

Organizations should emphasize the importance of identifying customer complaint factors and complaint intentions that crucially determine their business success. With respect to those who consider that complaints are worthless and not beneficial and management should consider enhancing mutual communication between service personnel and customers.

It is concluded that excellent service is a genuine key for a better future, for both customers and suppliers (Day, 2004). However, this can only be achieved with a profound knowledge of evolving customer needs. A functional CCM system will generate this knowledge, and such system should be implemented in every company regardless of its size, structure or products.

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Characterization of ZnS Quantum dot (q-dot) by Ultraviolet Visible (UV-VIS) Absorption Spectrum Studies & Comparison with CuO Nanocrystal

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ABSTRACT: Ultrasize ZnS quantum dots have been synthesized with (3-Mercatopropyl) trimethoxysilane as the capping agent by the all-aqueous procedure. The size of quantum dot by this method is in the range 4 nm to 10 nm. These quantum dots have been characterized by UV-Visible absorption spectrum. The absorption spectrum of synthesized quantum dots indicate a blue shift with decrease of size of quantum dot. Further UV-Visible absorption spectrum of quantum dot has been compared with that CuO nanocrystal.
[Mamun Mohanty, Aurobinda Acharya, Bairagicharan Panda, Selvaraju Balamurgan, Subhendu Pattnaik, Gourisankar Roy. **Characterization of ZnS Quantum dot (q-dot) by Ultraviolet Visible (UV-VIS) Absorption Spectrum Studies & Comparison with CuO Nanocrystal**. Journal of American Science 2011;7(1):348-351]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Quantum dots, UV-Visible spectrum, Blue shift

1. INTRODUCTION

Recent advances in producing highly luminescent quantum dots have led to the applications of quantum dots in imaging biological samples [1, 2] but the composition toxicity is a problem. Most of the quantum dots contain toxic heavy-metal elements such as Cd, Hg, Pb etc., which make them, unfit for some practical applications. It is well known that semiconductors are characterized by a band gap of the order of an electron volt (eV), between the valence band, which is fully occupied with electrons, and the empty conduction band. Hence under some circumstances they can emit radiation of a specific wavelength when electrons are injected into the material. This is called electroluminescence [3, 4]. The same thing can also happen when electrons are promoted from the valence to the conduction band by shining light of appropriate wavelength. Under these conditions, holes will be left behind in the valence band and when the electrons and the holes recombine, radiation with energy equal to the band gap will be emitted. This phenomenon is called photoluminescence[5, 6, 7]. For either of the abovementioned phenomena to occur the semiconductor must be of 'direct band gap' type, which is a requirement arising from momentum conservation in the process of light emission. Besides, for the emission of visible light of different wavelengths the band gap must have energy appropriate to the corresponding wavelength. This, in turn, requires that it should be possible to tune the band gap of the semiconductor. The commonly used semiconductor silicon is an 'indirect band gap' material with the magnitude of the gap rather small compared to the energy of light in the visible range. Hence it is not a light emitter. In contrast Cadmium sulphide (CdS) and Cadmium selenide (CdSe) are 'wide band gap' semiconductors [8, 9]. Hence these are more appropriate for light emission provided their band gaps can be tuned for emission of a particular colour of light. CdSe fulfills this requirement when prepared in the nanoform. When CdSe nanoparticles prepared in different sizes are suspended in a liquid and white light is shone on the test tubes containing these suspensions, each test tube emits light of a different colour depending on the size of the nanoparticle suspended in it. This clearly indicates that the band gap of CdSe changes depending on the size of the nanoparticle. in fact, the smaller the size the larger is the band gap of the material. As a consequence of these even materials that are not emitters of light in their bulk form start emitting in their nanoform. This is often referred to as "band gap engineering" or "quantum size effect". Thus silicon, the base material of electronics technology, can be made to emit light in its nanoform.

Figure 1: Fluorescence in different-sized CdSe quantum-dots

One of the most favourite q-dots of biology are CdSe nanoparticles We can make CdSe quantum dots in three or four different sizes and illuminate them with the same light when they would fluoresce in different colours. as shown in the figure-1.

This property can also be used for reporting about different functioning of the cell as shown in the figure 2. But to do that they need to be made water-soluble so that they can enter the cell as inert objects. To enable them to report on different zones or regions of the cell they can be attached to different biological molecules, which in turn can then attach to the cell membranes or pass through them reporting on their activity. With this technique in mind, Scientists have succeeded in detecting cancer cells at an early stage; which is a big achievement.

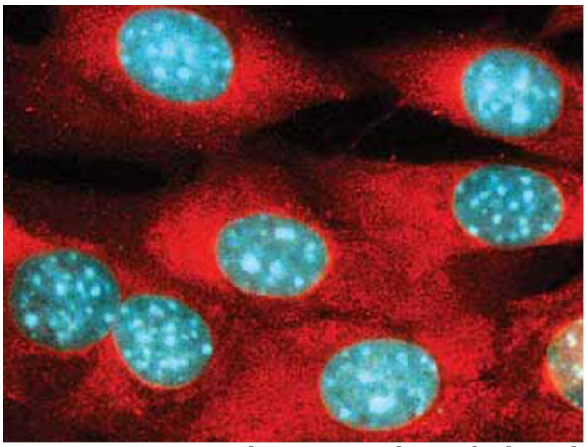

Figure 2: Quantum-dots in visualising biological processes

However, more investigation is called for before putting the use of q-dots into a common practice for any diagnostic activity, just to ascertain that their use does not cause any toxicity. One way of making these quantum dots nontoxic is to coat them with some inert material. Nonetheless, the possibilities with quantum dots in imaging, diagnostics and site-selective drug delivery into cells are exciting and extensive. There is the possibility that they will emerge as biosensors, bio-analytical agents, etc., with multifarious uses. The many faceted monitoring of cells using q-dots of varying sizes and coatings, to report on what is happening at different sites in the cell is still at a developmental stage.

Recently Evident Technologies developed a type of non heavy metal quantum dots, which are harmless in that way - ZnS quantum dots. ZnS quantum dots prepared earlier was unstable and big in size so that they get easily disintegrated and settle down. In order to achieve small size quantum dots we have synthesized ultra fine ZnS quantum dots through one-step aqueous procedure with (3-mercatopropyl) trimethoxysilane as a capping layer. The quantum dots prepared by this method are very stable and highly luminescent. After preparation of the sample we have studied UV-VIS spectrum of ZnS quantum dots with the help of Lambda 35 UV-Visible Spectrometer and compared with that of CuO nanocrystal.

2. EXPERIMENTAL WORK

The experimental work has been done in Raman Research Institute, Banglore.

2.1 PREPARATION OF ZnS SEMICONDUCTOR QUANTUM DOTS

ZnS quantum dots were synthesized directly with (3-Mercatopropyl) trimethoxysilane as the capping agent by the all-aqueous procedure .The prepared ZnS quantum dots are highly stable and exhibit photoluminescence. The size of the quantum dots obtained by this method was in the range between 4 to 10nm. The experimental procedure as follows 0.04 M of aqueous zinc nitrate was prepared by dissolving 1.18988 g of zinc nitrate in 100 ml deionized water, and 0.02 M of aqueous sodium sulfide was prepared by dissolving 0.5136 g of sodium sulfide in deionized water. For a sample with the MPS:Zn:S ratio of ½ : 2 :1 , 0.04 mmol of MPS was dissolved in 41 ml of deionized water and stirred for 5 min with a magnetic stirrer. 2ml of the 0.04 M zinc nitrate solution was added to it in a drop wise fashion with a constant stirring for 10min.The mixture was then titrated with tetrapropylammonium hydroxide until the pH value of the reaction mixture reaches 12 followed by the rapid addition of 4 ml of 0.02 M of sodium sulfide solution. Now, the reaction mixture is left for 5 min without any disturbance to form ZnS quantum dots before adding another 2 ml of 0.04 M of zinc nitrate with constant stirring for 5 more minutes. The final

suspension was clear and colourless. The obtained quantum dots suspension was quenched at 0^0 C and then stored at 4 ^{0}C, to stop further reactions.

Samples with different MPS:Zn:S ratios have been synthesized in the same manner but in varying amount of MPS which results in formation of quantum dots of varying size. All the above reactions were carried out under temperature environment of 200 ^{0}C to 300 ^{0}C in the laboratory.

3. UV-VISIBLE SPECTROMETER

We have used the Lambda 35 UV- Visible Spectrometer which is a versatile spectrometer operating in the ultraviolet and visible spectral ranges. Lambda UV-Visible spectrometer is scanning double beam spectrometer uses two light sources, a deuterium lamp for ultraviolet light and a halogen lamp for visible light as shown in the figure 3. The mirrorM1 is raised to permit radiation from the lamps to strike source mirror M2.The radiation from the source lamp is reflected from source mirror M2 through an optical filter to passes through a slit and hits a diffraction grating (monochromator) which can be rotated allowing a specific (single) wavelength to be selected. Appropriate optical filter on a filter wheel assembly located on the beam path to pre filter the radiation before it enters the monochromator. The radiation is dispersed at the monochromator to produce a spectrum. The rotational position of the grating effectively selects a segment of the spectrum, reflecting this segment through exit the slit 2 to mirror M3.This slits provide a spectral selectable band pass of 0.5,1,2 or 4nm. From the mirror M3 the radiation is reflected onto a beam splitter, which allows 50% of the radiation to pass onto the plane mirrorM4, and reflects on a filter wheel assembly located on the beam path to pre filter the radiation before it enters the monochromator. The radiation is dispersed at the monochromator to produce a spectrum. The rotational position of the grating effectively selects a segment of the spectrum, reflecting this segment through exit the slit 2 to mirror M3.This slits provide a spectral selectable band pass of 0.5,1,2 or 4nm. From the mirror M3 the radiation is reflected onto a beam splitter, which allows 50% of the radiation to pass onto the plane mirror M4, and reflects 50% of the radiation onto the plane mirror M5. Mirror M4 focuses the radiation beam to the sample cell. The beam then passes through a convex lens onto the photodiode detector. Mirror M5 focuses the radiation beam in to reference cell. . The beam then passes through a convex lens onto the photodiode detector.

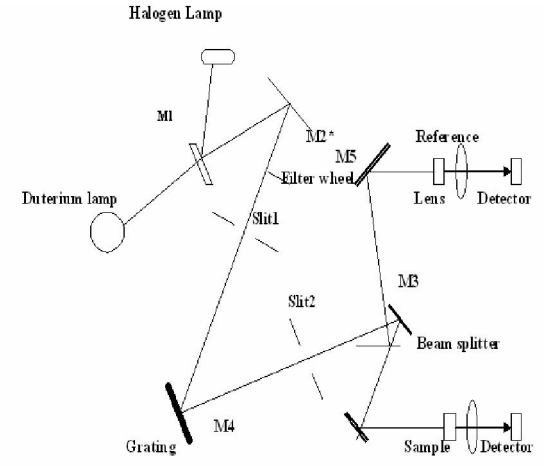

Figure 3: Block Diagram of UV-Visible Spectrometer

The advantage of double-beam operation is the better stability and allows reference to be measured and corrected in real time and fast scanning is done. The grating monochromator used here is a holographic concave grating with 1053 lines /mm in the center. Photodiodes are used as detectors. The optical path length in the sample compartment is 121mm. This spectrometer can be operated in a ambient operating temperature of 15^o C to 35^o C and humidity range of 20% to 80% without condensation. The power requirements to operate this spectrometer are about 100V to 240 V AC, frequency of 50/60Hz.

4. RESULTS AND DISCUSSION

The absorption spectrum of synthesized quantum dots as shown in figure 4 shows a blue shift as the quantum dot size decreases. This is because of the fact that when geometry of surface of quantum dots changes, the band gap energy changes. In case of small size quantum dots the band gap will be energetically larger. Such a quantum dot is blue shifted reflecting the fact that electron should fall to a greater distance in terms of energy thus producing a radiation of shorter wave length. Had it been a case of larger size quantum dot they would been red shifted producing a radiation of larger wave length.

Further we have studied the Ultra-Violet absorption spectrum of CuO nanocrystal. Unlike the spectrum of ZnS quantum dots where there is a absorptance with Increase in wavelength upto 330 nm wavelength and then after remains constant, there is a wave like pattern of UV-Visible absorption spectrum in the case of CuO nanomaterial in the wave length region 500 nm – 800 nm as shown in the figure 5.This shows that the absorption band of CuO nanomaterial.

In case of CuO nanocrystal the UV-VIS spectrum (figure 5) absorption band shows a clear blue shift.

This optical phenomenon indicates that these nanocrystals show quantum size effect. The absorption band of CuO nanocrystal is in the wavelength range of 300 nm to 400 nm and the hump in the broad absorption range (500 nm to 800 nm) is due to surface plasma.

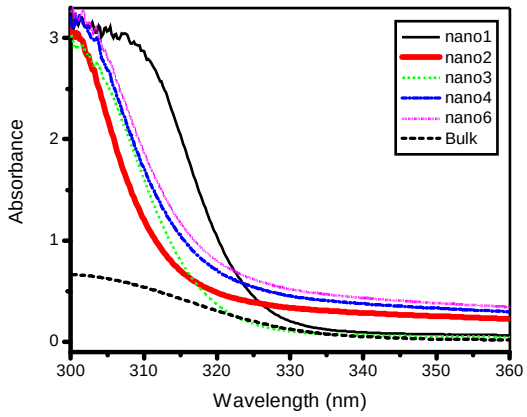

Figure 4: UV-Visible absorption spectrum of ZnS quantum dots

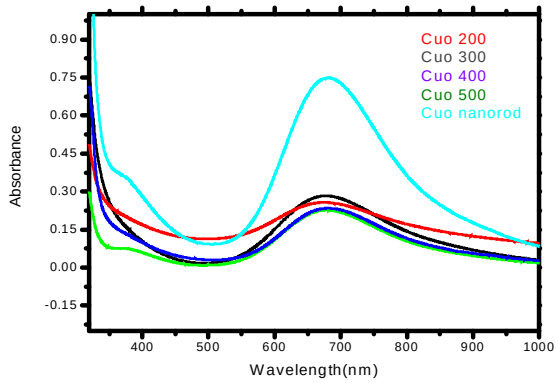

Figure 5: UV-Visible absorption spectrum of CuO nanocrystals

5. CONCLUSION

Thus we conclude that the absorption spectrum of synthesized quantum dots shows a blue shift with decrease of quantum dot size. Further the optical phenomenon indicates that CuO nanocrystals shows quantum size effect. The absorption band of CuO nanocrystals also shows blue shift.

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12/7/2010

Therapeutic and Protective Effects of Biphenyl Dimethyl Dicarboxylate (DDB) and Silymarin in Human Infected with HCV and in Carbon Tetrachloride Induced Hepatitis in Rats

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Abstract: This investigation aimed to evaluate the therapeutic activity of pure and commercial products of Biphenyl Dimethyl Dicarboxylate (DDB) and Silymarin in humans suffering from HCV and therapeutic and protective effects of Carbon tetrachloride (CCL4) induced liver damage in rats. *Humans* were divided into two groups: Group I: Normal controls (N=20), and group II: Patients suffering from chronic HCV infection; which were subdivided into two subgroups: A, ten patients received Silymarin 140 mg twice daily for one month and B, twenty patients received DDB 10 pilules (15 mg) twice daily for one month. Samples from control and treated groups were collected and obtained serum was analyzed for Aspartate aminotransaminase (AST), Alanine aminotransaminase (ALT), Alkaline phosphatase (ALP or Alk.ph.), Gamma Glutamic transaminase (GGT) and Serum bilirubin (total and direct). In addition, the effect of DDB or Silymarin administration on the mentioned biochemical parameters was measured. Other experiment was conducted in which rats were divided into nine groups, each group comprising of six rats. All rats except the control group were subjected to administration of Silymarin or DDB in pure and commercial products, before and after treatment with CCL_4. All serum samples of rats were subjected to liver function tests including: (AST), (ALT), (ALP.) and serum bilirubin as well as kidney functions tests including: blood urea and serum creatinine. Histopathological examination of liver tissues was also performed. The results revealed that DDB improved liver functions in patients suffering from HCV infection, while Silymarin showed insignificant alteration for the same parameters. The raw and commercial products of Silymarin or DDB were significantly improved liver, kidney functions and the histopathological changes after induction of CCL_4 toxic hepatitis in rats. Administration of DDB (commercial) for one month to patients suffering from chronic viral hepatitis resulted in a rapid decrease in serum transaminases, especially ALT. Treatment of rats by pure and commercial DDB for 7 days showed improvement in acute hepatocellular necrosis or hepatitis-associated hepatocellular damage caused by carbon tetrachloride. Administration of commercial Silymarin for one month was largely ineffective in patients suffering from viral hepatitis. The results of 7 days treatment by pure and commercial products of Silymarin in rats showed protection of liver tissue. Silymarin has an antioxidant effect. In rats Silymarin increased the level of total protein which indicates hepatoprotective activity as results of accelerate of regeneration process and production of liver cells. Obtained histopathological study confirmed the results of biochemical studies. It is concluded that a superiority and efficacy of DDB over Silymarin in normalizing the liver enzymes and serum bilirubin (total and direct) levels were achieved after treatment of humans suffering from HCV.

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Keywords: DDB, Silymarin, humans, HCV, Rats, CCL_4, hepatotoxicity.

1. Introduction:

DDB is synthetic analogue of schizandrin C, one of the active components isolated from *Fructus schizandra*, a traditional oriental medicinal plant (1). DDB has a beneficial effect on elevated liver enzymes and histopathological changes (2); it was used successfully for treatment of cases of chemically induced hepatitis (3 and 4). Silymarin therapy decreases complications, hastens recovery, and shortens hospitalization in patients with acute viral hepatitis (5). Silymarin prevent hepatic fibrosis through suppression of inflammation and hypoxia in the fibrotic liver (6). In Egypt, the HCV type 4 infection is a known viral infection.

Pegylated Interferon combined with ribavirin has been reported as the curative therapy in about 48% of patients with type 1 and 4 (7). Most of patients have elevated liver enzymes and bad general condition with progressive liver cell failure. However, drugs like Silymarin and DDB were noticed to decrease liver enzymes with improvement of the general condition of the patients (8-9-10and11). The exact mechanism of these drugs is unknown (12).

2. Materials and methods:

Drugs:

Biphenyl Dimethyl Dicarboxylate (DDB pilules) is as commercial product which was obtained from Beijing Union Pharmaceutical Factory, China. Pure DDB powder was obtained from Arabic Company of Medicinal Plants (Mebaco, Egypt).

Silymarin (Marriagon® capsules) was obtained from Alpha Chem. Advanced Pharmaceutical Industries Co. (ACAPI), Egypt.

Pure Silymarin powder was obtained from Arabic Company of Medicinal Plants (Mebaco, Egypt).

Carbon tetrachloride (CCL4) was obtained from Egyptian company for chemicals and pharmaceuticals (ADWIA).

All kits were obtained from Biodiagnostic Company, Egypt. Gamma Glutamic transaminase (GGT) was obtained from Quimica Clinica Aplicada S.A, Spain.

Human group: Fifty subjects were included in this work. They were divided into two groups: a- Thirty patients suffering from chronic HCV infection. Age ranged from 30-55y (13 females and 17 male). b- Twenty normal controls. Age ranged from 21-45 y (9 females and 11 males). Patients with diabetes, hypertension, renal failure and pregnant females or any organ failure were excluded.

Animal group: Fifty four Sprague Dawley albino rats male or female weighing 100–120 g were obtained from animal house unit of the National Research Center. The animals allowed free access to water and fed on uniform standard diet formula according to Rogers (1979) (13).

Methods:

Experimental design:

i-Human study:

Human were divided into two groups:

Group I: twenty normal controls.

Group II: patients with chronic HCV infection; were subdivided into two subgroups:

A. Ten patients received Silymarin 140 mg twice daily for one month.

B. Twenty patients received DDB 10 pilules (15 mg) twice daily for one month.

Thirty patients and twenty normal controls were subjected to the following laboratory investigations, AST, ALT, Alkaline phosphatase, GGT and serum Bilirubin. The effect of treatment by DDB or Silymarin on the mentioned biochemical parameters were measured in patients groups.

ii-Animal study:

Curative and hepatoprotective effect of Silymarin and DDB was studied. Carbon tetrachloride was used to induce hepatotoxicity in rats. Each drug was given on the 3rd day, for 7 days and the blood samples (3ml) were collected on 10th day; except for the 2nd group they were collected on 3rd day. The drug doses in the forthcoming work were calculated according to Paget and Barnes (14). Fifty four rats were divided into nine groups, each group comprising six rats:

Group 1: Placebo group of 6 rats received a single oral dose of one ml saline for 10 days.

Group 2: Six rats received a single oral dose of CCL_4 dissolved in paraffin oil (1:1) v/v ratio. Each rat received 0.25 ml of this solution once and blood samples were taken after 3 days according to the method reported by Janakat and Al Merie (15).

Group 3: Six rats received a single oral dose of CCL_4 dissolved in paraffin oil (1:1) v/v ratio. Each rat received 0.25 ml of this solution once and blood samples were taken after 10 days.

Group 4: Six rats received a single oral dose of CCL_4 dissolved in paraffin oil (1:1) v/v ratio. Each rat received 0.25 ml of this solution once, and then pure Silymarin was given. Each rat received 2.2 mg/ml water according to the method reported by EL-Shenawy (16) for 7 successive days.

Group 5: Six rats received a single oral dose of CCL_4 dissolved in paraffin oil (1:1) v/v ratio. Each rat received 0.25 ml of this solution once, and then the commercial product of Silymarin (Marrigon) was given. Each rat received the equivalent of 2.52 mg/ml water of Silymarin for 7 successive days.

Group 6: Six rats received a daily oral dose of pure Silymarin. Each rat received 2.2 mg /

ml water for 7 successive days, and then a single oral dose of CCL_4 dissolved in paraffin oil (1:1) v/v ratio was given. Each rat received 0.25 ml of the latter solution once.

Group 7: Six rats received a single oral dose of CCL_4 dissolved in paraffin oil (1:1) v/v ratio. Each rat received 0.25 ml once, and then pure DDB was given. Each rat received 10 mg/ ml water according to the method reported by Qing and Liu (17) for 7 successive days.

Group 8: Six rats received a single oral dose of CCL_4 dissolved in paraffin oil (1:1) v/v ratio. Each rat received 0.25 ml of this solution once, and then the commercial product DDB was given, each for 7 successive days.

Group 9: Six rats received a daily oral dose of pure DDB. Each rat received 10mg/ml of water for 7 successive days, and then a single oral dose of CCL_4 dissolved in paraffin oil (1:1) v/v ratio was given. Each rat received 0.25 ml of the latter solution once.

All rats are subjected to the following investigations: • Liver function test: AST, ALT, Alkaline phosphatase, and serum total bilirubin. • Kidney function test: blood urea and serum creatinine.

Laboratory investigations methods:

Serum ALT and AST were determined according to Reitman and Frankel (18), Serum alkaline phosphatase was measured according to Belfield and Goldberg (19), Serum Total Bilirubin was determined after Walter and Gerade (20). Serum Urea Nitrogen was measured according to Henry et al. (21). Serum creatinine was measured according to Bartles et al. (22). Serum ã glutamyl transferase (GGT) activity was measured according to Shaw (23).

Histopathological examination:

Tissue specimens from liver and kidney of treated and control rats were fixed in 10% neutral buffered formalin solution. The fixed specimens were trimmed, washed and dehydrated in ascending grades of alcohol, cleaned in xylene, embedded in paraffin then sectioned (4-6 micron) and stained with hematoxyline and eosin according to Bancroft et al (24). The sections were thereafter examined and photographed using a microscope at a magnification power of 200 X The degree of hepatic injury was estimated using an ordinal scale modified from Palaa and Charbonneau (25). According to the following table.

Histological Grading of Liver Injury:

Grade	Description
0	No apparent injury by light microscopy
I	Swelling of hepatocytes
II	Ballooning of hepatocytes
III	Lipid droplets in hepatocytes
IV	Necrosis of hepatocytes

Statistical analysis:

Data obtained were statistically analyzed using ANOVA test and t-student test using SPSS 14 (2006) (26).

3. Results and Discussion

HCV is one of the viruses that affect the liver causing hepatic injury leading to acute inflammation followed by its chronic form, which may be complicated by cirrhosis and hepatocellular carcinoma (27). Commercial product of Silymarin was leading to insignificant drop in liver functions and studied parameters in patients, when it was compared with their levels before treatment (Figs 3 and 4). Silymarin was largely ineffective in patients with viral hepatitis (28). Silymarin treatment for HCV over 125 days did not significantly change ALT, AST and GGT levels (29). Furthermore, the use of Silymarin did not significantly affect serum HCV RNA and ALT levels in patients (30).

The obtained data indicates that treatment by commercial product of DDB has a powerful effect in the improvement of the liver function parameters in patients suffering from HCV (Figs 5 and 6). Concerning DDB results, the results nearly similar to those recorded by Liu (31) , Li (32) , Shimabukuro (33) and Akbar et al. (34) who mentioned that administration of DDB for 2 weeks or more decreased the average blood level of ALT. The patients with chronic hepatitis C, B, or steatohepatitis, with persistently elevated ALT when treated with DDB, ALT can be rapidly normalized in most of the cases and remained normal during treatment (35). Furthermore, Li et al. (36) mentioned that Schisandrin from Schisandra fruits were able to scavenge hydroxyl radicals and superoxide anions much stronger than that of vitamin C and vitamin E.

It was reported that the administration of DDB to patients suffering from HCV caused a decrease in serum bilirubin blood level after treatment for three months (37), this going with the results

obtained as shown in (Figs. 7-12). It was worth noting that the administration of CCL_4 to rats followed by administration of pure Silymarin (group 4) or commercial one (group 5) on the third day for 7 successive days leads to a remarkable decrease of at least 30% to 50% in AST, ALT, Alk. Ph., Bilirubin and Creatinine, but insignificant decrease in serum urea level was recorded. Hence, it could be concluded that the administration of pure or commercial Silymarin exerted an anti-inflammatory effect against CCL_4.

This observation is in concordance with the findings revealed that Silymarin prevented all the changes observed in CCL_4 hepatocirrhotic rats which could be attributed to both its antioxidant and membrane stabilizing action (38) or as result of membrane stabilization, neutralization of the free radical and immune modulation occurred in experimental animals (39). Concerning the effect of Silymarin in the present study, the obtained results agreed with what reported that treatment with Silymarin at 25 mg/kg body weight to Wistar albino rats after the induction of liver damage by D galactosamine, was able to normalize the serum levels of ALT, AST, ALP, total bilirubin, lactate dehydrogenase, total cholesterol, triglycerides, albumin, total protein levels (40). Also, Silymarin significantly reduced the liver toxicity in rats indicated by decline of the levels of AST, ALT and ALP activities in serum as compared to toxicated rats (41).

Concerning administration of pure Silymarin before CCL_4 in rats (group 6) in the present work, a remarkable decrease in blood level of ALT, Alk. Ph., bilirubin, creatinine and urea was shown. Silymarin exerted a protective effect through decreasing CCL_4 induced lipid peroxidation and hepatotoxicity in mice (42). Approximately similar results were reported (16).

Administration of pure DDB (group 7) or commercial one (group 8) after CCL_4 on the third day for 7 successive days revealed a remarkable and significant ($P<0.001$) decrease in liver enzymes (AST, ALT and Alk. Ph.), bilirubin, and creatinine. Serum urea showed insignificant reduction after DDB treatment .These results indicated the efficacy of DDB as anti-inflammatory liver cell agent in induced liver damage. Moreover, findings are in concordance with the results proved that DDB is of a beneficial effect on damaged liver resulting from CCL_4 and thioacetamide administration. Also, it is highly effective in normalizing the liver functions with very low side effects (43) (32) (44) and (45).

The administration of pure DDB before CCL_4 ingestion (group 9) caused improvements of the hepatocytes and consequently lowered the blood level of liver enzymes. It was proved that pretreatment of rats with DDB ameliorate the reduction of liver glycogen and blood glucose in chemical induced hepatitis. Also the serum level of ALT, AST, and Alkaline phosphatase were significantly lowered compared with the CCL_4 intoxicated rat groups (43) and (46). This result is nearly similar to that reported by Ip et al. (47) who mentioned that the treatment of animals with CCL_4 caused drastic increases in both plasma alanine aminotransferase (ALT) and Sorbitol dehydrogenase (SDH) activities in mice. However pretreating mice with Schisandrin B or C (DDB) regimen significantly ($P<0.001$) improved the CCL_4 -induced toxicity condition (hepatoprotective effect). The observed hepatoprotective action against CCL_4 is due to the ability of DDB to maintain hepatic mitochondrial glutathione redox status under oxidative stress condition (48).

Pharmacological study showed that DDB increases liver protein and glycogen synthesis and has an inducing effect on the cytochrome P-450 enzyme system (32). The mechanisms of DDB hepatoprotection effect is functioning as a potent antioxidant agent when it is used in the treatment of viral and chemically induced hepatitis (48). Effects of DDB may protect hepatocytes by stimulating the hepatic mitochondrial reduced glutathione (GSH) antioxidant system via activation of GSH related enzyme. GSH works with the antioxidant enzymes, such as S-glutathione peroxidase, glutathione Stransferases, and glutathione reductase, in combating reactive oxygen species and maintaining cellular glutathione status, in this process, the maintenance of mitochondrial glutathione status was critical for cell survival (49) and (50).

As shown in Figs. (12 and 13) it could be concluded that strong correlation between the laboratory analytical results in serum liver enzymatic activities of patients and rats before and after treatment with pure and commercial products of Silymarin and DDB, it could be concluded also that the percent of changes of comparison between patients and rats before and after treatment with Silymarin on liver enzymes was proved to be of no concept. On the other hand, administration of DDB revealed its potent therapeutic and protective effect on both rats and humans.

The liver and kidney specimens of the control group (group 1) was normal regarding their size and colour. Histological examination of liver showed normal hepatic lobules associated with normal histological structure of the portal triad as shown in figure (15). The liver revealed grade (0). Also, kidney's parenchyma appeared with normal histological structure (Fig.21). These results were in complete agreement with those reported by Das et al. (51).

Group (2) which exposed to CCL_4 and examined after 3 days revealed necrobiotic changes of hepatocytes including vascular degeneration, nuclear pyknosis and necrosis as well as narrowing of hepatic sinusoids and hyperplasia of Kupffer cells. In addition portal triads showed fibrous connective tissue proliferation and hyperplasia of bile duct and hepatic injury appeared as grade (IV) which illustrated in figure (16). CCL_4 is one of the most commonly used hepatotoxic agents in experimental study of liver diseases (52). Furthermore, CCL_4 is biotrasformed by cytochrom P-450 in liver to produce highly reactive trichloromethyl free radical. This radical, in presence of oxygen generated by metabolic leakage from mitochondria, causes peroxidation of lipids membrane which led to loss of integrity of cell membranes and damage of hepatic tissue(53). Moreover, changes in structures of the endoplasmic reticulum and other membranes cause loss of metabolic enzyme activation, reduction of protein synthesis and loss glucose-6-phosphatase activation which over all leads to liver damage (54) and (55). On the other hand, Kidney of the same group showed swelling of tubular epithelial lining especially the proximal convoluted tubules and coagulative necrosis of some renal tubules as clearly evident in figure (22).

Liver specimens of rats belonging to group (4) that received pure Silymarin powder after being treated with CCL_4 showed ballooning degeneration of hepatocytes and single cell necrosis. Moreover, hyperplasia of bile duct by forming numerous numbers of new bile ducts was clearly apparent in figure (17). This hepatic injury appeared as grade (II). This showed that Silymarin has a hepatoprotective effect by improving the appearance of the hepatocytes. These findings are in concordance with that reported by Barbarino et al. (56) who mentioned that Silymarin is beneficial in reducing the damage of hepatocytes (57) , added that, Silymarin is advantageous for regenerating the normal function of the liver, after being exposed to CCl_4 hepatotoxication. Moreover, Mourelle M. (38) and Muriel P. (58) proved that Silymarin prevented the increase in lipid peroxidation caused CCL_4. Kidney specimens of the same group revealed mild swelling of tubular epithelial lining in comparison with those of the 2nd group Fig. (23).

Liver specimens of rats, belonging to group 5 which were exposed to CCL_4 followed by treatment with the commercial Silymarin capsules (Marriagon®) for 7 days, showed swelling of hepatocytes and narrowing of sinusoids. Moreover, focal areas of coagulative necrosis were also seen. The liver specimens appeared as grade (III) as clearly demonstrated in figure (18).

Liver specimens of the rats belonging to group (7) that received pure DDB material for 7 consecutive days after CCL_4 treatment showed mild swelling of hepatocytes accompanied by narrowing of hepatic sinusoids. The liver specimens appeared to be grade (I). DDB induced more hepato-regenerative effect than Silymarin as the tissue injury appeared as grade (I) as shown in figure (19). Histological examination showed normal histological structure in kidneys as evident in figure (24). These results are in agreement with that reported by Fu T. and Liu G (43) who proved that DDB has extremely beneficial effects on both damaged and normal hepatocytes. The same was held true with the findings of (17) who mentioned that DDB is able to directly and indirectly antagonize certain damage in the hepatocytes. Moreover, (4) mentioned that DDB administration caused improvement in the histopathology examinations of the chemically injured liver.

Liver specimens of rats belonging to group (8) that were exposed to CCL_4 then treated with commercial DDB product for 7 days showed mild swelling of hepatocytes and narrowing of sinusoids as depicted in figure (20). The liver specimens appeared as grade (II).

Humans

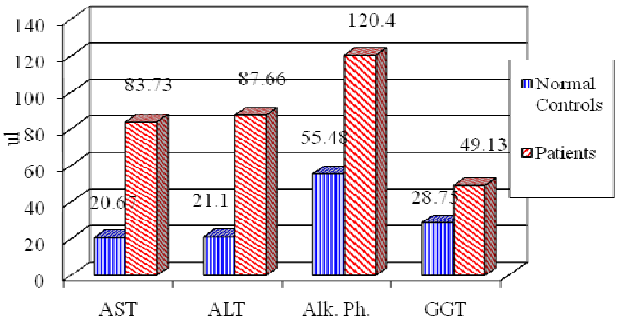

Figure 1: Liver Enzymes Parameters of Normal Controls and Patients.

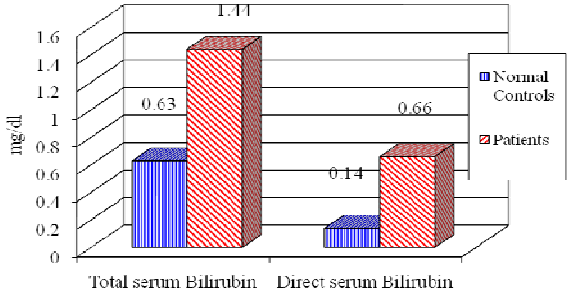

Figure 2: Serum Bilirubin of Normal Controls and Patients

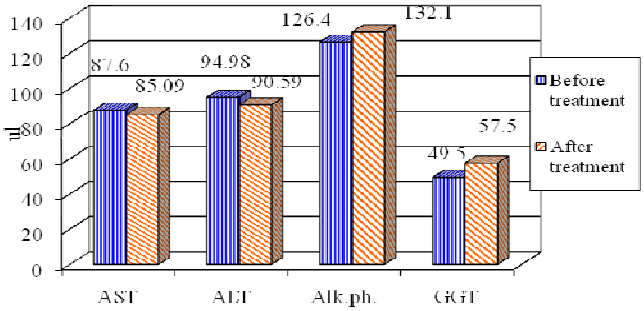

Figure 3: Effect of Silymarin on Liver Enzymes

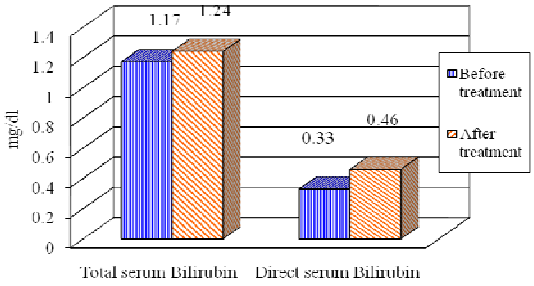

Figure 4: Effect of Silymarin on Serum Bilirubin

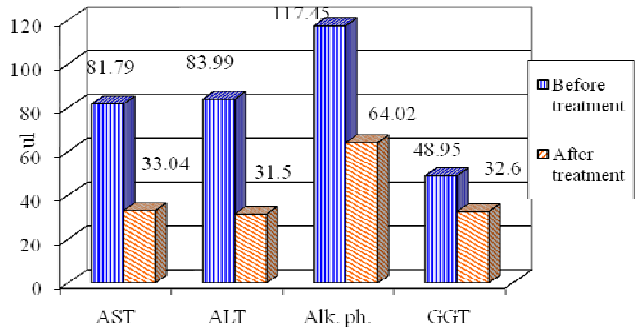

Figure 5: Effect of DDB on Liver Enzymes

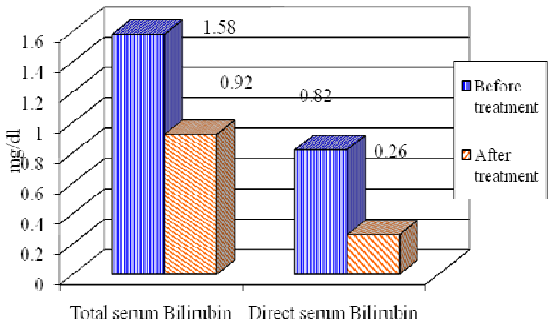

Figure 6: Effect of DBB on Serum Bilirubin.

AST = Aspartate aminotransaminase (u/l).
ALT = Alanine aminotransaminase (u/l).
Alk.ph. = Alkaline phosphatase (u/l).
GGT = Gamma glutamic transaminase (u/l).

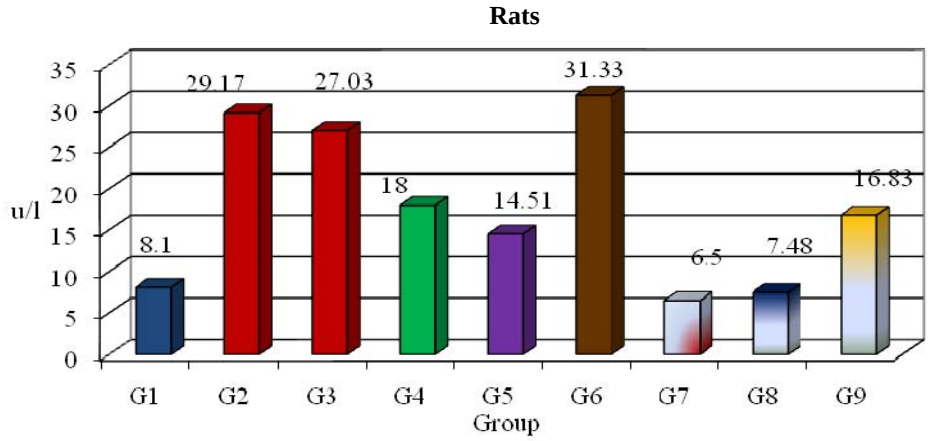

Figure 7: Comparative Effects of Silymarin and DDB on hepatitis induced by Carbon Tetrachloride in Rats for AST u/l.

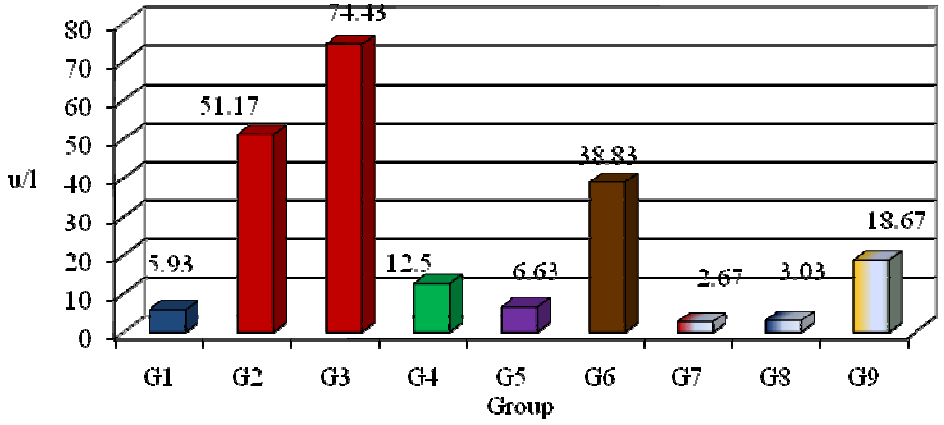

Figure 8: Comparative Effects of Silymarin and DDB on hepatitis Induced by Carbon Tetrachloride in Rats for ALT u/l.

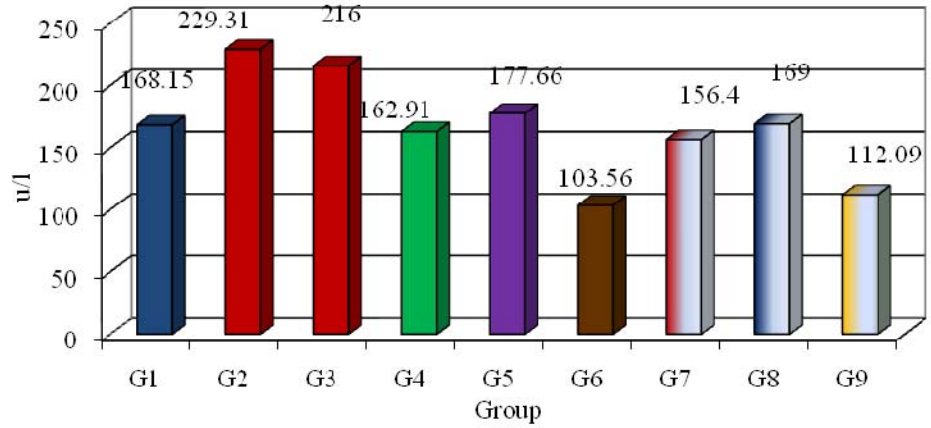

Figure 9: Comparative Effects of Silymarin and DDB on hepatitis induced by Carbon Tetrachloride in Rats for Alk.ph. u/l.

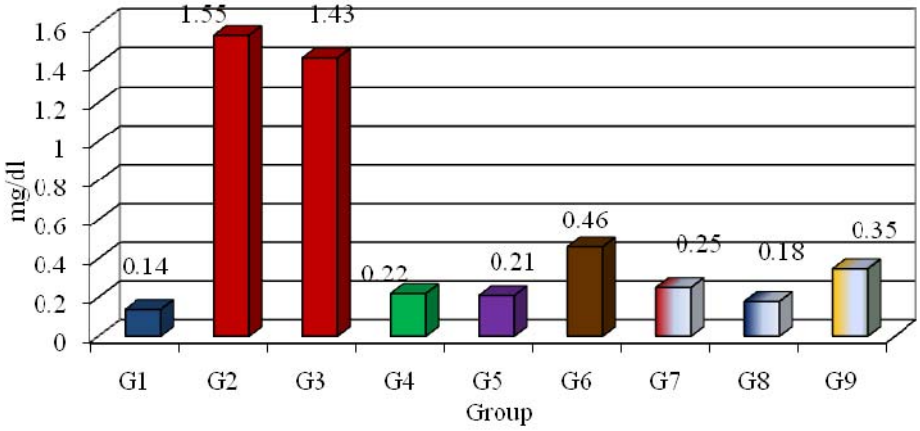

Figure 10: Comparative Effects of Silymarin and DDB on Hepatitis induced by Carbon Tetrachloride in Rats for Serum Bilirubin (mg/dl).

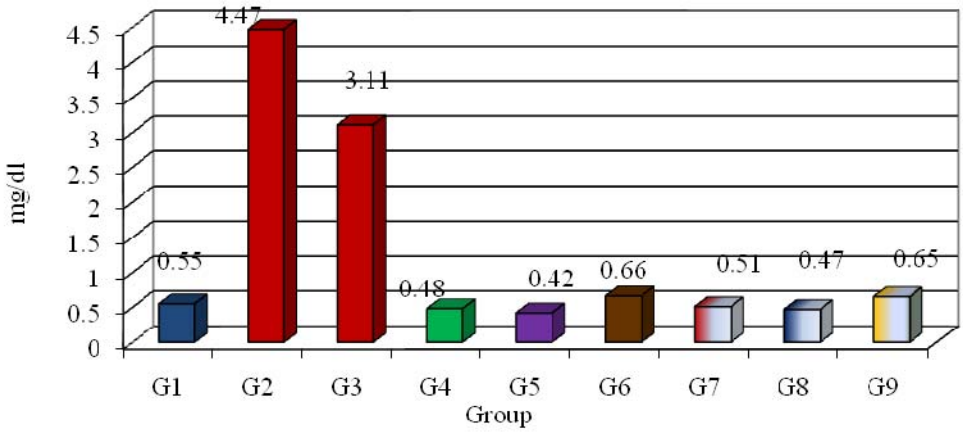

Figure 11: Comparative Effects of Silymarin and DDB on Hepatitis induced by Carbon Tetrachloride in Rats for Serum Creatinine (mg/dl).

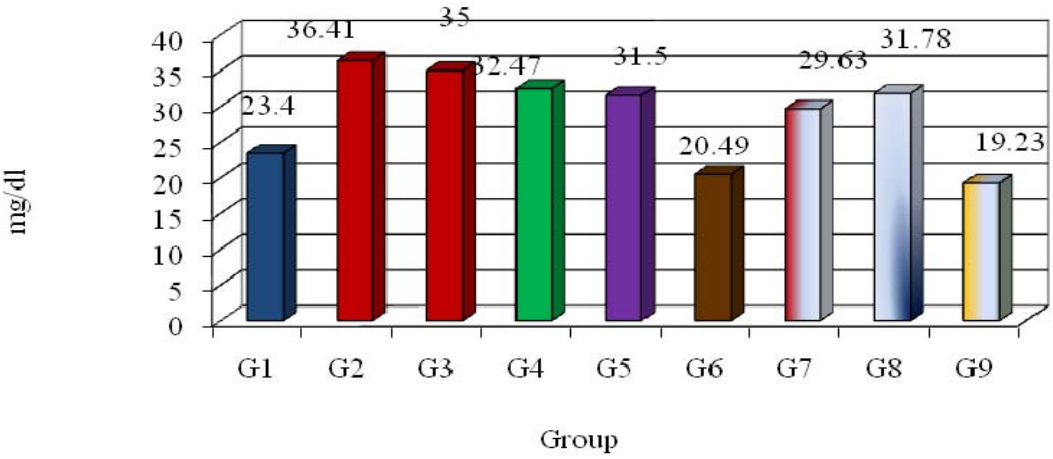

Figure 12: Comparative Effects of Silymarin and DDB on Hepatitis induced by Carbon Tetrachloride in Rats for Serum Urea (mg/dl).

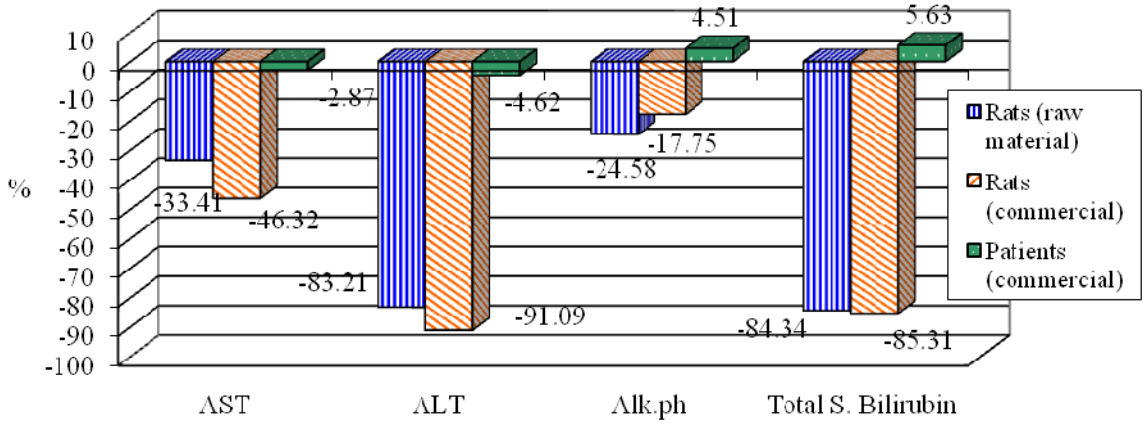

Figure 13: Impact of Treatment with Silymarin on the Measured Liver Parameters in Rats and Patients.

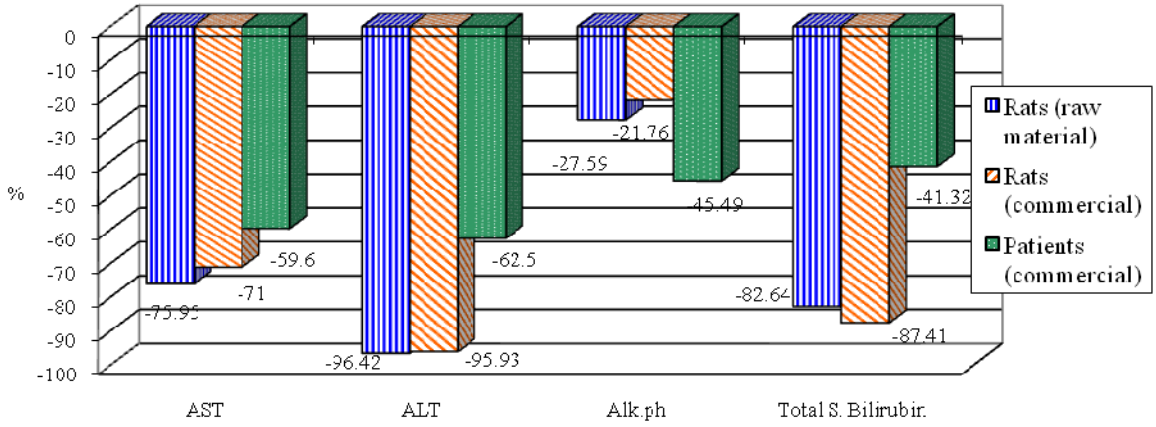

Figure 14: Impact of Treatment with DDB on the Measured Liver Parameters in Rats and Patients.

Liver

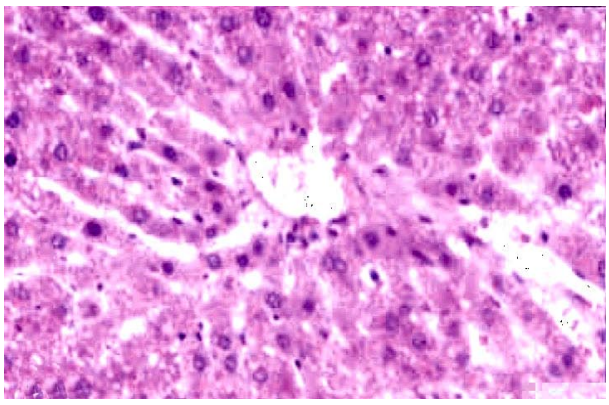

Figure (15): control group (grade 0) (H&E X200).

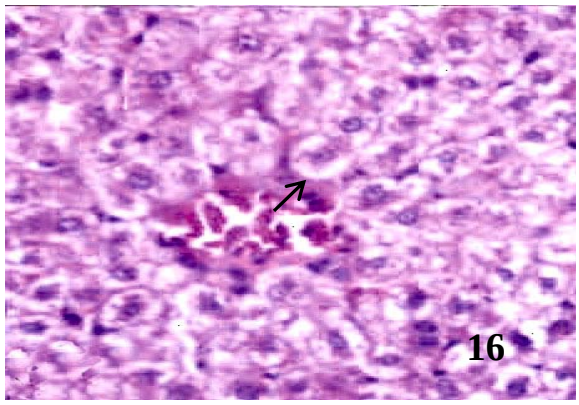

Figure (16): CCL_4 group (grade IV) (H&E X200).

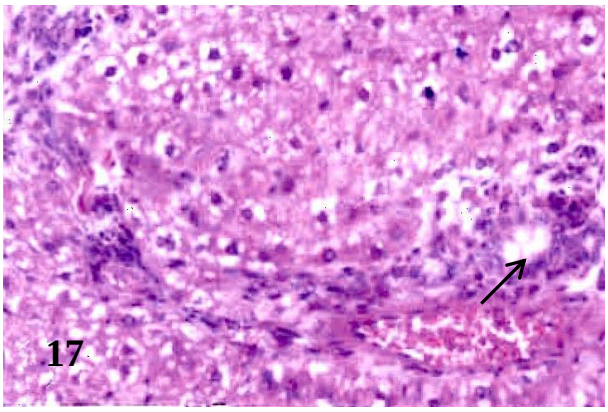

Figure (17): Group four (raw material of Silymarin) after exposed to CCL_4) (grade II) (H&E X200).

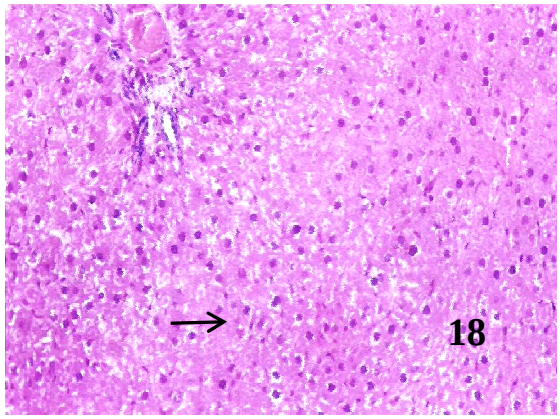

Figure (18): Group five (commercial product of Silymarin) after exposed to CCL_4 (grade III) (H&E X200).

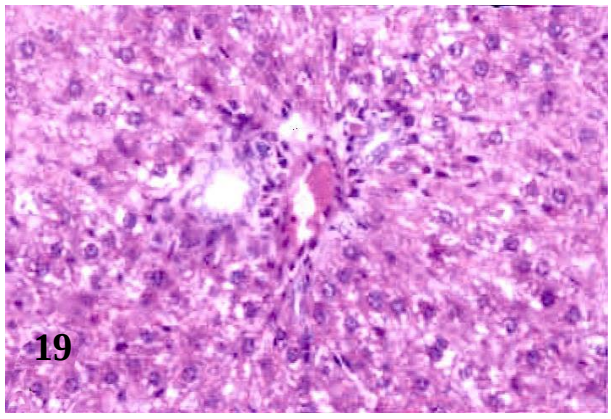

Figure (19): Group seven (raw material of DDB) after exposed to CCL_4 (grade I) (H&E X200).

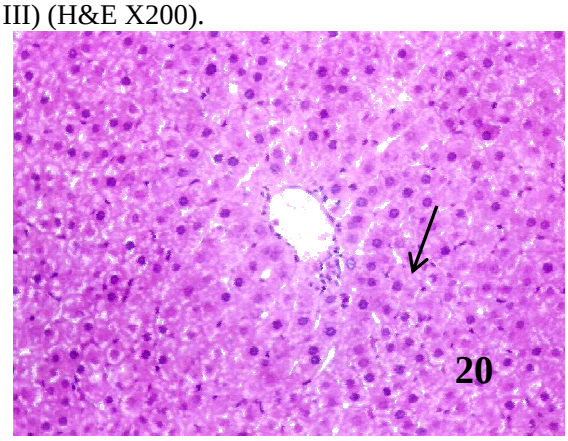

Figure (20): Group eight (commercial product of DDB) after exposed to CCL_4 (grade II) (H&E X200).

Kidney

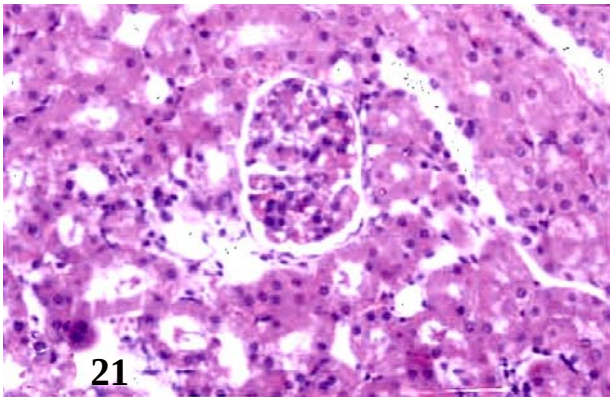

Figure (21): Control group, normal histological structure of it is parenchyma (H&E X200).

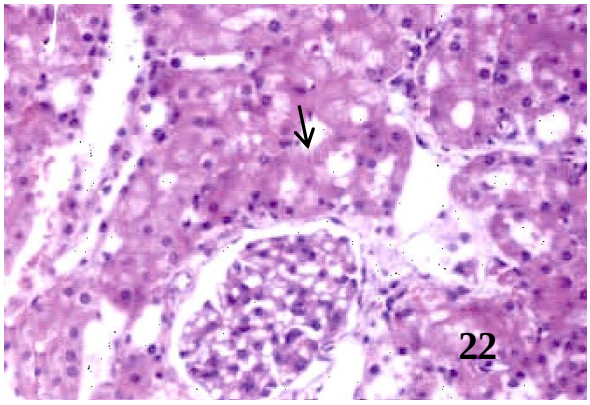

Figure (22): CCL_4 group, swelling of tubular epithelial lining (H&E X200).

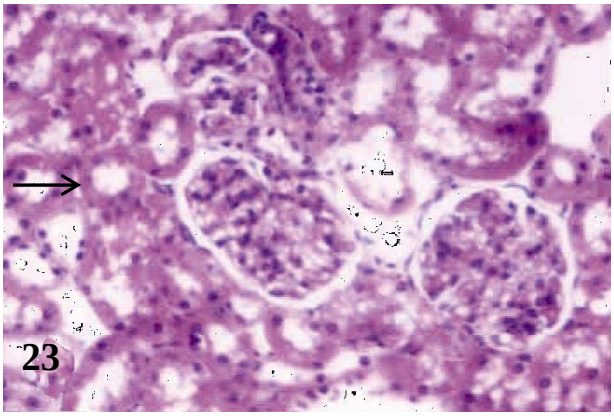

Figure (23): Group four (raw material of Silymarin) after exposed to CCL_4, mild swelling of tubular epithelial lining (H&E X200).

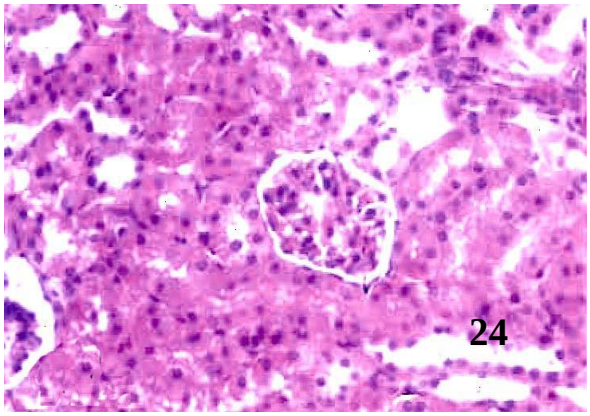

Figure 24: Group seven (raw material of DDB) after exposed CCL_4, normal histological structure (H&E X200).

4. Conclusion:

1- DDB was improved liver functions as regards to AST, ALT, ALP., serum bilirubin and GGT in patients suffering from HCV infection.
2- Silymarin has insignificant effect on the liver enzymes and serum bilirubin in patients suffering from HCV infection.
3- The raw and commercial materials of Silymarin and DDB were significantly treated the liver and kidneys after CCL_4 induced toxic hepatitis in rats.
4- Raw material of DDB and Silymarin is better than their commercial product in their action on treatment of CCL_4 induced hepatitis in rats.
5- Commercial product of DDB is better than commercial product of Silymarin as regard the action on liver enzymes and creatinine in rats.
6- Raw material and commercial products of DDB and Silymarin were improved the histopathological changes in CCL_4 induced hepatitis in rats.
7- The rats might be considered as a good representative model for humans in researches tackling liver infections.

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Design and Manufacturing of Parabolic Trough Solar Collector System for a Developing Country Pakistan

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Abstract: Pakistan's thirst for electric power has been constantly rising over the years because of population growth, increase in industrial activity and failure of other resources for producing enough energy to meet its growing energy demand, particularly in the remote areas where energy is most needed. Pakistan is basically an energy deficient society and now going towards extreme energy crisis. Moreover, with current demand growth at 8 % annually, Pakistan will have to add 4000 MW to its existing capacity by the year 2018. Pakistan is rich in renewable energy resources; particularly solar energy has a special relevance in Pakistan due to high availability of Sun radiations at an average rate of 4.5-6 kwh / m^2 / day. The purpose of this research is to reduce the cost of conventional power plant by focusing on simplifying the design of collector structure to achieve a high reflecting quality and tracking precision, using available cost effective components, minimizing field construction requirements, and by utilizing the advantages of design engineering and equipment specifications as per environmental impact at feasible locations in most remote and energy starved areas of Pakistan. Most of the area of Pakistan lies in sunny belt of the earth with the sun shine of 6 – 8.5 hours daily having the greatest amount of radiant energy more than 90% of solar radiation, which comes as direct radiation because of the limited cloud coverage and clear sunny weather is experienced 250 to 300 days a year. Different concentrating technologies have been developed or are currently under development for various applications. The Parabolic Trough Solar Collectors system will undoubtedly provide within next decade a significant contribution to efficient, economical, sustainable renewable and clean energy supply to developing countries with positive effect on environmental activities. The collector materials will be used considering conversion efficiency, abundance of the material, low cost structures, ease of application, expected lifetime, and the availability of space at the collection site. Available sites in Pakistan desert can theoretically cover the whole electricity demand of the country. A small configuration system like 25KW can lead to 100MW by scale up as sub unit of larger power plants. This will be the first step to fulfill the energy demand of Pakistan, which has become essential for our economic revival.

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Keywords: Species richness; beta-diversity; taxonomic diversity; forest

1. Introduction

Renewable energy sources have also been important for humans since the beginning of civilization. Pakistan is facing severe energy challenges – indigenous oil and gas reserves are running out, energy demand is rapidly increasing, gap between demand and supply is growing, concerns about secure supply of energy are increasing and fuel cost is rising at an unprecedented rate. Pakistan has 84% dependence on oil and gas to meet its primary energy demands. It has an electrical power generation system with more than 19,404 MW of installed power, with 63.97% being generated by thermal resources. The emissions from power plants cause smog in cities and various undesired health complications for our children and us. Rapid progress in solar energy technology around the world went unnoticed in Pakistan for several years due to frequent shuffling of decision makers as well as absence of solar resource analysis. Several countries have planned solar energy participation in the active energy matrix. Because of the threats associated with dependence on use of oil and gas for generating power, now it is the time to evaluate commercial aspects of this technology in Pakistan and venture into this field by taking lead. It costs us nothing to get it--just free sunny days all over this country. There are almost no on-going costs; just build the plant and let it produce free power for well more than over 20 years because most of the area of Pakistan lies in sunny belt of the earth with the sun shine of 6 – 8.5 hours daily Fig 1.

Energy produced by sun is being used to produce heat and electricity and all essential requirements in a house like water heating, home heating, lighting, cooking and cooling in many parts of the world but not in Pakistan. Pakistan is presently in the process of developing various energy technologies. Solar option with its merits and demerits is also a promising technology of tomorrow and must be given its adequate place today so that we remain current with this technology once it is fully matured. The Solar system and the associated power with the sun cannot be effectively covered in few words. Currently, research in the field of solar power generation is very limited in Pakistan.

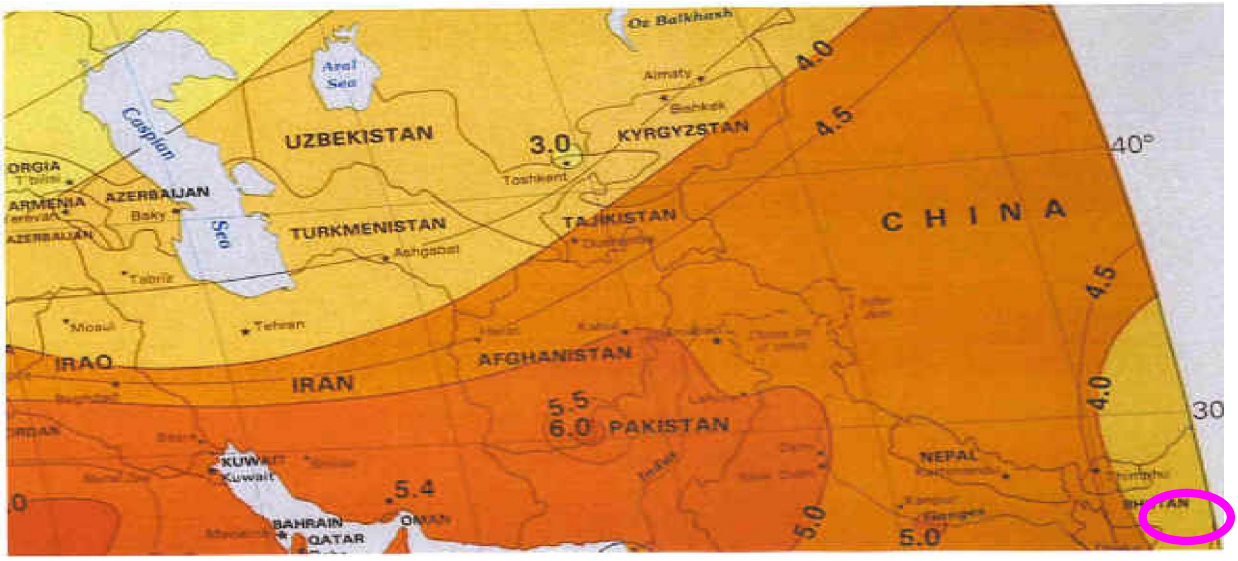

Fig.1. Solar insulation map of Pakistan (Courtesy of the Advanced Energy Group, http://www.solar4power.com)

2. Solar Thermal Power Technology

The idea of using mirrors to concentrate sunlight is not a new idea. It is said that, in 212 BC, Archimedes used polished bronze shields to focus sunlight, trying to set fire to wooden ships from the Roman Empire which were besieging Syracuse. Although we don't know whether this worked, the Greek navy recreated the experiment in 1973 and successfully set fire to a wooden boat at a distance of 50 meters. Leonardo da Vinci proposed the use of concave mirrors to heat water on an industrial scale. Parabolic trough solar technology is the most proven and lowest cost large-scale solar power technology available today, primarily of the nine large commercial-scale solar power plants that are operating in the California Mojave Desert. These plants developed by Luz International Limited and referred to as Solar Electric Generating Systems (SEGS), range in size from 14 - 80 MW and represent 354 MW of installed electric generating capacity. More than 2,000,000 m^2 of parabolic trough collector technology has been operating daily for so many years. The Luz collector technology has demonstrated its ability to operate in a commercial power plant environment like no other solar technology in the world. A number of new parabolic trough projects are currently in varying stages of project development around the world, some of these will include thermal energy storage. Energy Information Administration, Renewable Energy Annual (1996), Charles Smith Technology Review, (July 1995), Pilkington Solar International: (1996)

The three most promising solar thermal technologies are the parabolic trough, the central receiver or solar tower, and the parabolic dish. But in this paper only parabolic trough collector will be focused as being the proven and reliable technology. It has been successfully demonstrated in the Californian desert for two decades using commercial parabolic trough technology and steam turbines, achieving an annual field availability of 99 %. Pilkington Solar International: (1996)

2.1 Parabolic Troughs

A parabolic trough solar collector is designed to concentrate sun rays via parabolic curved solar reflectors onto a heat absorber element – a "receiver" – located in the optical focal line of the collector. Solar collectors track the sun continuously. The key components of a parabolic trough power plant fig.2 are mirrors, receivers and turbine technology.

The receiver consists of a specially coated absorber tube, which is embedded in an evacuated glass envelope. The absorbed solar radiation warms up the heat transfer fluid flowing through the absorber tube to almost 400°C. This is conducted along a heat exchanger in which steam is produced, which then generates power in the turbines.

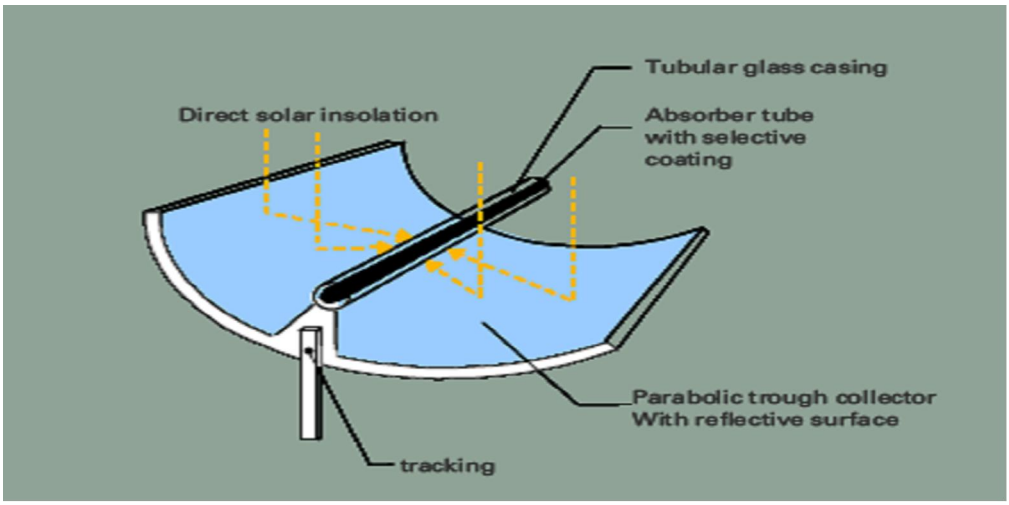

Fig.2: Parabolic Trough Concept

2.2 Tracking the Sun

Tracking is particularly important in solar energy collection systems that work under concentrated light. The parabolic concentrators always require the orientation towards the sun. By tracking the sun from sunrise to sunset, the parabolic collectors concentrate the sun's radiation with their parabolic mirror facets on the absorber tubes along their focal line to collect the heat. The mirrored troughs face the sky and direct sunlight to a large metal and glass receiver in the middle of trough that holds circulating oil. The thermal efficiency of a parabolic trough solar collector depends on the accuracy with which the collector follows sun. Tracking system consists of 0.25kw, 685 rev/min, 8-pole AC motor with electromechanical brake and a 463:1 high – reduction helical gearbox. Stephenlas Vegas, Nevada, Renewable EnergyAccess.com, Naidoo, P., Nelson Mandela Metropolitan University, (2005)

2.3 Land

Parabolic trough plants require a significant amount of land that typically cannot be used concurrently for other uses. Parabolic troughs require the land to be graded level. Pakistan has a range of desert in remote area where such small configuration power units are needed. In general, a parabolic trough solar power plant in a good resource reigns requires approximately 5 acres (20,000 m^2) per MW of plant capacity. Kearney,D., and C. Miller, (January 15, 1988). A study for the state of Texas shows that land use requirements for parabolic trough plants are less than that those of the most of other renewable technologies (wind, biomass, hydro) and also less than those of fossil when mining and drilling requirements are included. Contract No. 500-89-001, San Diego, CA :(December 2, 1991), (July, 1995), ISBN 0-9645526-0-4

2.4 Wind

The performance and structural design of solar field are impacted by high winds. The solar field is not designed to operate at winds of more than15.64 meter/sec; consequently, high-wind sites limit the performance potential of the solar plant. Moreover, wind forces dictate the collector structural design. Since the structure constitutes about 40% of solar field costs, it is important to optimize this component. Price, H.; and Kearney, D. (1999)

2.5 Mirrors

The glass mirrors are one of the most reliable components in the parabolic trough solar collectors. The mirrors concentrate the sunlight more than 80 times on a metal absorber pipe in the line of focus. San Diego Regional Renewable Energy Study Group, (August 2005). Mirror mounting ceramic pad is given special emphasis to cater for the problem caused by differential thermal expansion between the mirror and the pad. Mirror breakage due to high winds has been observed near the edges of the solar field where wind forces are high. These mirrors are manufactured in Pakistan with the size limitations of manufacturing plants. Each mirror fig 3 is supported on the structure at four points on its backside.

Design of Collector Structure

Factors considered in the construction of the parabolic trough solar collector include stability and accuracy of the parabolic profile, optical error tolerance, and method of fabrication, cost, material availability and strength constraints. H.

Guven,Technical Brief in Journal of Solar Energy Engineering, Vol. 116, No. 3, pp. 164-166, (1994) proposed a Parabolic Trough Solar Collector (PTSC) design approach that differentiates between developed and developing nations, where design objectives are not limited to maximizing thermal efficiency but must also favor cheaper, labor-intensive design and production techniques. Elements of this approach, which partitions the PTSC design problem into a macro-level stage dealing with the reflector, receiver and tracking system and a micro-level stage in which the subsystems are integrated, were employed in this research. Deviations included pre-selecting the rim angle based on parabolic-rib material constraints and selection of the receiver glass envelope diameter based on availability of glass tubing.

This research work produced a 4 m long parabolic trough solar collector assembly Fig.4. The three of these assemblies are placed in one row to be operated with just one drive mechanism. This meant that 12m long collectors per drive could be constructed which would meet the high optical requirements, at all stages of operation, when sunlight falling onto the 5.07 m wide aperture has to be concentrated onto an absorber diameter. The horizontal wind loads and the low degree of permissible distortion are relevant to the design of a suitable steel-glass structure. The frame structure of parabolic trough collector is made up of four steel ribs. The cross-section is reinforced using diagonal struts and end frames, providing the support points for the mirror of thickness of 5mm.

Fig. 4 Frame structure of parabolic trough collector design and developed by researchers

Consequently, a 12 m long collector assembly in one row consists of a reinforced of 3 parabolic reflectors. Fig 4 shows at every 4m, a support for the pivotal mounting of the collector elements is located and fixed to a drive pylon, which bears the dead weight of the collectors and the horizontal wind load. The drive pylons are fitted with hydraulic drives which enable the total 12 m long collectors to track the current position of the sun, satisfying the high degree of precision being (0.04 degrees) required. Renewable Energy Annual (1996), US Department of Energy, Washington, DC 20585, USA; April (1997) The entire steel structure of 4m long for the loop of 24m lengths is manufactured at site in Pakistan. This saves the transportation expenses of huge assembly of steel structure to the power plant site. As the collectors are optical devices, and a high degree of geometric precision is required – which, as a welded construction, is only achieved at great effort and cost – the individual components are manufactured using the degree of precision typical to the steel construction industry and the final collector geometry was then achieved when accurately assembling in special jigs on site fig 6. This enables geometric precision to be achieved, almost to the nearest millimeter, necessary for optical performance. Energy Information Administration, Renewable Energy Annual (1996).Reflector mirrors of 924.38×1333.33mm in total 18 mirrors per collector assembly each with a length of 4 m and width of approx 5.07 m are installed on a surface area.. Each 4m long elements of collector are joined together to an approximately 24m long collector unit, tracking the sun by using hydraulic drives. The one loop is made up of three of these collectors which are arranged in the field in a north-south direction. Therefore, the collector field reflective surface area of approximately 240 m².

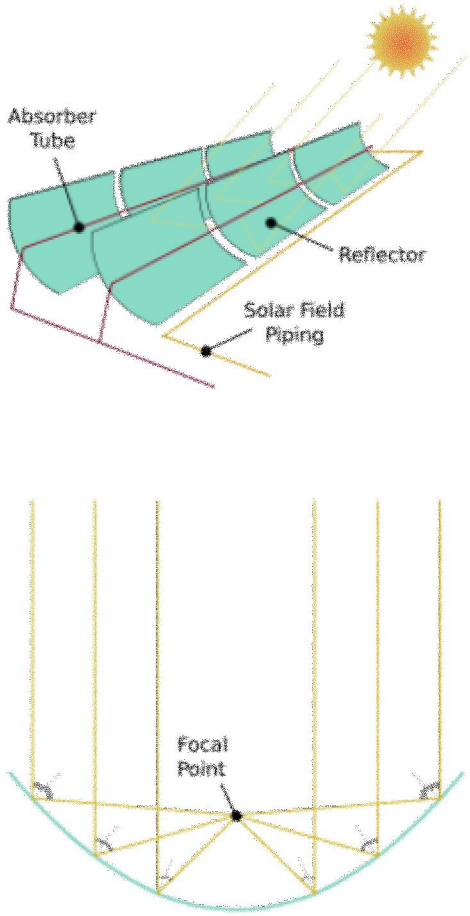

Fig.5. A diagram of a parabolic trough solar farm (top), and an end view of how a parabolic collector focuses sunlight onto its focal point.

The thermal heat transfer medium synthetic oil is pumped through the individual loops of 12 Solar Collector Assemblies (SCAs) Fig.5 that are arranged in two parallel rows of 6 SCAs each and is heated by about 100°K as it flows through a loop, by means of the concentrated solar radiation. The heat transfer medium thus heated to a temperature of about 400°C is then pumped to the steam generator. Energy Information Administration, Renewable Energy Annual (1996).

Fig.6 Structure of parabolic trough collector showing four steel ribs and Pylon

The Heat Collecting Element (HCE) fig 7 is a steel absorber tube of 7cm in diameter, which is coated with black chrome .The absorber tube, is surrounded by a glass envelope. The space between the steel tube and the glass is evacuated to limit heat losses from the absorber tube to the surrounding environment.

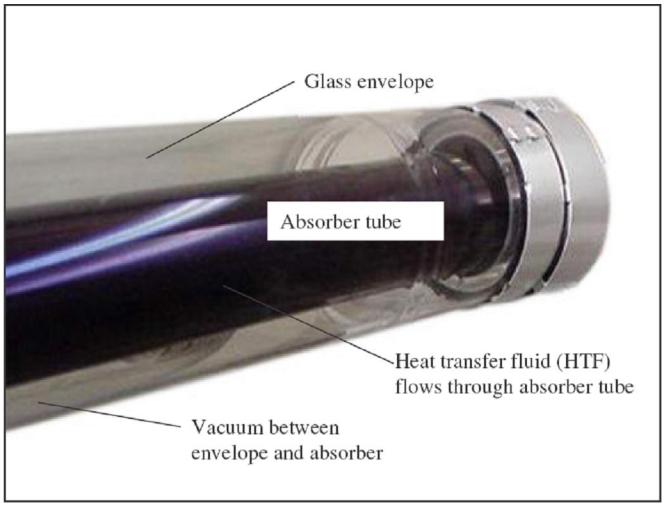

Fig.7 Heat Collecting Element (source : Solel UVAC, 2004)

The focused radiant energy from the sun is absorbed through the HCE and transferred to a heat transfer fluid (HTF), which is synthetic oil such as a mixture of biphenyl and diphenyl oxide (Therminol VP-1) that is pumped through each HCE tube. The heated HTF is pumped back to the power plant, where it becomes the thermal resource for steam generation in the power cycle. Cologne, Germany: (June 1994).

3.1 Technical Requirements of Parabolic Trough Solar Collector

Longitude	**Between 62 and 75 degrees East**
Latitude	Between 24 and 37 degrees North
Structure	Steel frame with mirrors supported arms
Aperture Width	5007milli meter
Focal length	1001millimers
Length per collector	4000millimeters
Length of Solar Collector Assembly	24000millimeters(two rows of 12000mm)
Wind load	39m/s
Rim angle	90°
Drive	Hydraulic
Heat Collecting Element	Evacuated tube
Interconnect	Rotating joints
Receiver technology	Parabolic trough solar field
Receiver absorptivity	0.96
Receiver emittance	0.20
Mirror reflectivity	0.93%
Mirror size	1333.33*924.38mm
Selective surface	Black chrome
Piping heat loss	10w/m ^{2}
Net out put	22kw
Errection method	On site simple assembly
Heat collecting element (HCE) pipe diameter	0.70mm
HCE length	4000mm
Concentration ratio	16.7

3.2 Power Plant Size

Increasing plant size is one of the easiest ways to reduce the cost of solar electricity from parabolic trough power plants. Studies have shown that doubling the size reduces the capital cost by approximately 12-14%.Pilkington Solar International: (1996) Status Report on Solar Thermal Power Plants. Report ISBN 3-9804901-0-6. The increased manufacturing volume of collectors for larger plants drives the cost per square meter down. Secondly, a power plant that is twice the size will not cost twice as much to build. Thirdly, the O&M costs for larger plants will typically be less on a per kilowatt basis. For example, it takes about the same number of operators to operate a 10 MW plant as it does for a 400 MW plant. Naidoo, P., Nelson Mandela Metropolitan University,(2005).

4. Latest Trends in Renewable Solar Energy Field

Chinese government supports the development of concentrating solar power (CSP) technology strongly, to change the energy-intensive and environment-burdensome economical development way, through renewable energy for sustainable electricity generation. Qu Hanga,_, Zhao Juna, Yu Xiaob, Cui Junkui, Renewable and Sustainable Energy Reviews 12 (2008) 2505–2514 . Recently solar thermal absorption cooling has again aroused researchers' interest in the development of high temperature solar receivers, double effect chillers, and advanced control The Center for Building Performance and Diagnostics (CBPD) at Carnegie Mellon University has carried out research on solar thermal absorption cooling and heating to assess the feasibility of this technology through installation, testing, modeling, and evaluation of a new system with an advanced system configuration using recently available parabolic solar receivers. Ming Qu a,*, Hongxi Yin b, 1, David H. Archer c, 2 Solar Energy 84 (2010) 166–182. Solar adsorption cooling machine, where the reactor is heated by a parabolic trough collector (PTC) and is coupled with a heat pipe (HP). This reactor contains a porous medium constituted of activated carbon, reacting by adsorption with ammonia. A. El Fadar a, A. Mimet a,*, A. Azzabakh a, M. Pérez-García b, J. Castaing, Applied Thermal Engineering 29 (2009) 1267–1270 . An experiment platform of a parabolic trough solar collector system (PTCS) was developed for thermal power generation, and the performance of the PTCS was experimentally investigated with synthetic oil as the circulate heat transfer fluid (HTF). The solar collector's efficiency with the variation of the solar flux and the flow rate of the HTF was identified. The collector efficiency of the PTCS can be in the range of 40%–60%. It was also found that there existed a specified delay for the temperature of the HTF to response to the solar flux, which played a significant role in designing the PTCS. LIU QiBin1, WANG YaLong1,2, GAO ZhiChao1,2, SUI Jun1*, JIN HongGuang1 & LI HePing3Sci , China Tech Sci, 2010, 53: 52−56, doi: 10.1007/s11431-010-0021-8. The major direct use solar thermal market is China,

which leads the world by a long margin, followed by the United States, Germany and Turkey. Although the installed capacity of CSP, solar power generation is still small it has started to take off in the last two years, notably in Spain and the United States. Reportlinker PRWire 2009 - By Robert Miller (18.12.2009) 12:44:16. An optimal design procedure for internally insulated, carbon steel, molten salt thermal storage tanks for parabolic trough solar power plants. The exact size of the vessel and insulation layers and the shape of the roof are optimized by minimizing the total investment cost of the storage system under three technical constraints: remaining within the maximum allowable values of both temperature and stress in the steel structure, and avoiding excessive cooling and consequent solidification of the molten salt during long periods of no solar input. R. Gabbrielli, C. Zamparelli. J Sol. Energy Eng. (November 2009) Volume 131, Issue 4, 041001 . Intel Corporation (Intel) has reported that new contracts are in place to incorporate around 2.5 MW worth of new solar power projects at eight US locations in Arizona, California, New Mexico and Oregon. In addition, Intel announced it has renewed and increased by 10% its purchase commitments for renewable energy credits (REC) to more than 1.43 billion kW hours, more than 51% of its estimated 2010 US electricity use. Global Data's Power Research Views Announced Date: (Jan 25, 2010).

5. Conclusions

The cost, performance, and risk of parabolic trough technology are fairly well established by the experience of the existing operating parabolic trough plants. As the government cannot afford the cost of supplying electricity to the far-flung remote area of Pakistan, those could be provided power on the cheapest production cost by developing Solar Energy System. Solar energy technologies have great potential to benefit our nation. They can diversify our energy supply, reduce our dependence on imported fuels, and improve the quality of the air we breathe and stimulate our economy by creating jobs in the manufacturing and installation of Solar Energy Systems. In order to effectively mold the trend of society towards the use of solar energy product, it is imperative that indigenously produced solar products are of high quality with better engineering design, having high efficiencies and cost-effectiveness for average users.

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INFLUENCE OF CHOLINE CHLORIDE ON QUALITY AND STORABILITY OF PEACH FRUITS CV. EARLIGRANDE.

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ABSTRACT: The effects of preharvest foliar application of Choline Chloride (CC) on fruit quality of "EarliGrande" peaches at harvest and during cold storage at 1ºC temperature was investigated. CC was sprayed at concentrations of 0, 500, 1000, 1500 and 2000 mg/L at 30 days preharvest time (DPH). Fruit weight was increased by 500, 1000 and 1500 mg/L CC. At the same concentrations SSC/TA ratio was increased while, fruit acidity was decreased. Sugar, phenol and vitamin C content tended to increase by CC at harvest time. The combination of CC treatments at 1000 and 500 or 1000 mg/L and cold storage at 1ºC resulted in a reduction of weight loss (%) in two seasons, respectively. CC in combination with storage resulted in higher fruit firmness, SSC, SSC/acidity and total sugar and a reduction in fruit acidity in both seasons.
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Keywords: peaches, EarliGrande, Choline Chloride, fruit quality, storability.

INTRODUCTION

Peach [*Prunus persica* (L.) Batsch] is one of the most important fruit species, which cultivated widely worldwide. In Egypt, it is considered one of the most important and favorable five fruit crops. The total area of peach grown in Egypt reached (78840 feddan) most of them concentrated in the new lands, **78% of th**is area is in North Sinai (61500 feddan) (Egyptian Ministry of Agriculture 2008). The main cultivar grown in North Sinai is EarliGrande that considered early season cultivar **(Wahdan *et al.*, 2003)**. The total areas of peach produce about 376067 Tons (Egyptian Ministry of Agriculture 2008).

Average for peach growing is increasing in Egypt, but fragile storability due to rapid softening and price fluctuation from short harvest span is a problem. As a result of increasing, the supply of peach fruits some methods need to improved the fruit quality be developed to distribute this production over a long period of time during the season. From these methods using some chemical treatments and cold storage to prolong the shelf life of the fruits.

Choline Chloride (CC) increases fruit growth and coloration in peaches and cherries **(Sato, 1994). Benincore *et al.*, (2000)** reported that, the foliar application of CC with different concentration on apple trees increased fruit weight and SS contents especially at 500 ppm. Moreover, CC produced oblong fruits with low flesh firmness. Rare information is available about the effect of foliar application of CC on "EarliGrande" peach fruits at harvest time and during cold storage. Thereat, this study aim to effect of preharvest CC sprays on peach quality at harvest and after cold storage at 1ºC temperature.

MATERIALS AND METHODS

EarliGrande peach trees 8 years old budded to Meetghamr rootstock grown in a sandy soil at Abou-Sweer, Ismailia Governorate, Egypt. Chosen trees were spraying month preharvest time (first week of April) with 0, 500, 1000, 1500 and 2000 mg/L Choline Chloride (CC) in two successive seasons 2007 and 2008. All treatments were applied with a handgun to the run off. The experimental treatments were arranged in a randomized complete block design, where five treatments were conducted, with three replicates each of one tree.

Commercial ripe fifteen fruits from each treatment were hand harvested in the first week of May for studying both physical and chemical characteristics at harvest date. One hundred maturity fruits as another sample from each treatment were hand harvested in the same time. The fruits were transported the lap within one hour of harvest. Fruits were stored to eliminate defects. Sound fruits were cleaned by using soft brush and packed in foam plates in one layer (every plate contain five fruits) covered with perforated polyethylene sheet (thickness 14 µ). All plates from each treatment

were stored at 1ºC and 85-90% RH: A plate (contain five fruits) was used per replicate. Three replicates were used per treatment.

Fruits were sampled at Zero time and at 7 days intervals for studying both physical and chemical characteristics as follows:-

-Fruit weight and weight loss (g) evaluation: fruits were weighted individually after harvest, labeled and stored. At each sampling time (7days intervals up to 21days) the same fruits were reweigh. Weight loss was expressed as a percentage of the original fresh weight of fruit and calculated as the following equation:

Fresh weight loss % = [(initial weight-sample weight)/initial weight] ×100

-Fruit shape: fruit length, diameter and length/diameter ratio were evaluated. Firmness (N): It was measured on two sides of the fruit using Effegi penetrometer, soluble solids contents (SSC%) by hand refractometer, fruit acidity and vitamin C according to **A. O. A. C. (1985),** total sugars according to **Stewart (1974),** total soluble phenols by Folin and Ciocaltu colorimetric method **(A. O. A. C., 1985).**

The statistical analysis was done between all the effects, CC treatments and storage duration. The experimental design was complete randomized block with a factorial arrangement of CC treatments and storage duration **(Steel and Torrie, 1980)**. Analysis of variances and mean comparison (LSD at 5%) were performed with Co-Stat program version 3.

RESULTS

Fruit quality

Physical properties

Data in **Table (1)** clear that, Choline Chloride (CC) treatments increased the average weight of fruit with all concentrations except highest level (2000 ppm) which gave lowest weight (93.8 and 93.5 g) in both seasons, respectively.

Regarding fruit volume, data tabulated in the same table show that, CC at 500 ppm in both seasons significantly increased fruit volume. On the other hand, CC at 2000 ppm in both seasons also significantly decreased fruit volume. While, the other treatments no significant differences obtained between of them in both seasons.

Concerning, specific gravity of fruits, no significant differences were noticed between treatments of CC in both seasons.

In the first season no significant differences were noticed in fruit length between CC treatments and control, except CC at 2000 ppm which significantly decreased fruit length. While in the second one all treatments significantly decreased fruit length relative to the control. Fruit diameter was increased with treatments of CC, except CC at 2000 ppm in the first season and CC at 1000 and 2000 ppm in the second one decreased fruit diameter relative to the other treatments **(Table 1).**

Fruit length/diameter ratio decreased by CC treatments in comparison with the control in both seasons, except the treatment of CC at 1000 ppm in the first season slightly increased fruit length/diameter ratio relative to control. The highest reduction of ratio was obtained by CC at 500 ppm in the two seasons.

Regarding to fruit firmness, data in **Table (1)** also shows that, the increment in fruit firmness was obtained by CC at 2000 ppm in the first season and by CC at 1000, 1500, 2000 ppm in the second one. The lowest values of fruit firmness (2.15 and 2.76) were obtained by CC at 500 ppm compared to (3.12 and 3.63) by control in both seasons, respectively.

Chemical properties

Data in **Table (2)** showed that, no significant differences were noticed in fruit SSC between the different CC concentrations in the first season, while in the second one the treatment of CC at 2000 ppm only significantly increased fruit SSC relative to other treatments which no significant differences were found among them.

CC treatments resulted in decreasing fruit acidity in comparison with the control in both seasons, except CC at 2000 ppm which increased fruit acidity and gave highest value (0.95 %) relative to other treatments in both seasons. As a result of this trend, all treatments of CC resulted in increasing SSC/acid ratio compared with control, except CC at 2000 ppm which reduced SSC/acid ratio in both seasons.

Data in the same table, showed that, CC at 1000 and 2000 ppm significantly increased fruit content of vitamin C in both seasons while, no significant differences were noticed among other treatments in both seasons. The highest values (22.65 and 23.4 mg/ g) were found with 2000 ppm CC and lowest values (15.85 and 15.15 mg/ 100g) were obtained from 500 and 1500 ppm CC in both seasons, respectively.

Sugar content tended to increase by all CC treatments in the first season and by CC at 1000 ppm only in the second one gave highest value of total sugars in peach fruits (6.5 mg/100g) in comparison with the other treatments.

CC treatments increased peach fruits content of total phenols in both seasons. The highest values of phenols (75.2 and 76.7 mg/ 100g) were obtained with CC at 1000 ppm and 500 or 1000 ppm

in comparison with the lowest values (72.2 and 73.4 mg/ 100g) which obtained with CC at 500 ppm and control in both seasons, respectively.

Weight loss (%)

Data in **Table (3)** revealed that, interactions of Chholine Chloride (CC) × storage periods (SP) were significant in both seasons. In first season, the significant increase in weight loss was obtained from CC at 1500 ppm (3.39 %) relative to the control (2.72 %) and CC at 1000 ppm (2.31 %). No significant differences were noticed between other treatments. In the second one, no significant differences were noticed in weight loss between 0 ppm (2.86 %) and 1000 ppm CC (2.54), but significant differences were noticed between all treatments. The highest value of weight loss % (3.71) was obtained with CC at 2000 ppm while, lowest value (2.12 %) was obtained with CC at 500 ppm compared with control (2.86 %). In general, during storage fruit weight loss increased significantly. The highest losses were obtained at last week in both seasons.

Firmness

In both seasons, the interaction effects of CC × SP were significant for fruit firmness **(Table, 3).** In both seasons, also fruit firmness increased with all concentrations of CC relative to the control, which record lowest values (4.51 and 4.86 kg/cm^2) in both seasons, respectively. Fruit firmness significantly decreased during storage for three weeks and the lowest value was obtained after three weeks of storage in both seasons. The combination of CC treatments and 1ºC storage temperature resulted in higher fruit firmness than using the same temperature with Zero concentration of CC in both seasons.

Soluble solids contents (SSC)

The interaction effects of CC × SP were significant in both seasons. **(Table, 4)** CC treatments resulted in significantly increasing fruit SSC over control in both seasons. Highest values (10.62 and 10.53 %) were noticed with treatment of CC at 1000 ppm compared with lowest values (8.80 and 8.48 %) which noticed with control in two seasons, respectively. Concerning of storage periods, data tabulated in the same table revealed that, fruit SSC increased significantly during storage periods. The highest values were obtained at the end of storage period in both seasons.

The combination of CC treatments and 1ºC storage temperature gave higher fruit SSC than using the same temperature with Zero concentration of CC in both seasons.

Acidity

In first seasons, the interaction of CC × SP was not significant for fruit acidity (Table, 4), while it was significant in the second one.

CC at all concentration decreased fruit acidity in both seasons, except treatment of CC at 2000 ppm in second season only which gave value of acidity equal to control. However, fruit acidity significantly decreased during storage period in both seasons.

CC treatments in combination with SP resulted in lower fruit acidity during third and fourth week of storage at all concentrations in comparison with the control.

SSC/Acid ratio

From **Table (5)** it clear that, the interaction effect of CC × SP was significant for fruit SSC/acid ratio in both seasons. Fruit SSC/acid ratio was increased significantly by CC treatments and during storage from Zero time up to the end of storage in both seasons. The highest increments in the ratio (17.7 and 18.94) were obtained by CC at 1000 ppm in the first and second seasons, respectively relative to control which gave the lowest ratios (10.51 and 11.08) in both seasons. The increments in ratio during storage can be explained by the increases in SSC and decreases in acidity, in addition to increases of fruit weight loss as result of cold storage and CC treatments.

The combination of 1000 mg/L CC and SP resulted in an increase of SSC/TA ratio during all weeks of storage relative to other concentrations.

Vitamin C

Data tabulated in **Table (5)** revealed that, the interaction of CC × SP was significant for fruit content of vitamin C. CC treatments at all concentrations increased vitamin C in peach fruits in both seasons, except the treatment of CC at 1500 ppm in the second season only. The highest significant increases in fruit content of vitamin C (19.5 and 19.5 mg/100g) from CC at 1000 ppm in the two seasons compared with control (14.75 and 15.5 mg/100g) in both seasons, respectively.

Concerning storage period, slightly decreased of fruit content of vitamin C from Zero time till third week then, significantly decreased in the fourth week was obtained in the first season, while in the second one the decrease was slightly from Zero time up to the end of storage period. CC at 1000 mg/L in combination with SP resulted in higher fruit content of vitamin C than other treatments.

Total sugar

The interaction effect of CC × SP was significant for fruit content of total sugar in both seasons. All treatments of CC in both seasons significantly increased sugar, except the treatment of CC at 500 ppm in second season which no different from control. The highest values of total sugar (5.25 and 5.30 mg/100g) were obtained with CC at 1000 ppm in both seasons in comparison with the control which gave (4.56 and 4.79 mg/100g) total sugar in both seasons.

Concerning the effect of storage period it could be seen that, total sugar significantly increased as the time of storage was increased which reached a maximum values at values at the end of storage period in both seasons. The increase in sugar contents may be due to the higher weight loss of these fruits, as a result of which there might have been an increase in the concentration of sugar. The combination of CC treatments and storage period increased fruit content of total sugar in both seasons specially the treatment of CC at 1000 mg/L during all weeks of storage.

Total phenol contents

From **Table (6)** also, it is clear that the interaction effect of CC × SP was significant for total phenol content in both seasons. CC treatments at all concentrations and in both seasons slightly decreased total phenol content of peach fruits, except the treatment of CC at 500 ppm in the first season only which gave the highest value (77.0 mg/100g) compared with all treatments. During storage, fruit phenol content significantly decreased from Zero time up to the end of storage in both seasons.

Discussion

Concerning physical properties, similar results were obtained by **Benincore *et al*., (2000)** on apple, they found that, spraying of CC at 500, 1000 and 1500 mg/L at 18 days preharvest time (DBH) increased mean fruit weight in apple. In addition, **(Akihinco, 2000)** reported that CC increased fruit weight and produced oblong fruits with low flesh firmness in Masui Dauphino fig cultivar.

Regarding chemical properties the obtained results in harmony with those of **Kim *et al*., (2004)** who reported that, foliar application of CC on "Mibaek" peach increased SSC, Acidity and sugar content. The results concerning physical characteristics and chemical properties may be attributed to the effect of CC on improving photosynthetic rate **(Akihiko *et al*., 2000).** Loss of moisture from the fruits during storage period might explain the increases in fruit weight loss **(El-Shiekh and Wahdan, 2002 and Abd-El-Salam, 2010).** The foliar application of CC decreased fruit weight loss percentage. **Akihiko *et al*., (2000) and Kim *et al.*, (2004)** found that fruit SSC was reduced by CC on Fig and Peach. In addition, **Abd-El-Salam (2010)** reported that, peach fruit SSC percentage during cold storage at 1ºC was significant increased tell the end of storage period. **Akbudak and Eris (2003)** stated that, the increase in total sugar contents observed in peach fruits having minute quantities of starch mainly, resulted from the conversion of polysaccharides in the cell walls to sugar. An increase in total sugar content during cold storage of peach has been reported by **Soukar and Ladaniya (1999).**

Concerning fruit firmness, the obtained results in this concern are in agreement with those found by **Abd-Al-Salam, (2010)** who reported that peach fruit firmness significantly decreased as storage time was increased. A gradual decline in total phenols may be due to polyphenoloxidase oxidize total phenols in peach fruit during cold storage **(Cheng and Crisosto, 1995).**

Table (1): Effect of foliar application of Choline Chloride (CC) on physical characteristics of Peach fruits cv. EarlyGrande during 2007 and 2008 seasons.

Treatments	Fruit weight (g)	Fruit volume (cm)	Fruit specific gravity	Fruit Length (cm)	Fruit Width (cm)	Fruit Shape index	Fruit Firmness
	2007 Season						
Control	113.65 b	126.1 ab	0.90 a	6.10 a	6.05 ab	1.01 ab	3.12 a
CC at 500 ppm	126.6 a	144.5 a	0.88 a	6.15 a	6.40 a	0.96 c	2.15 b
CC at 1000 ppm	117.65 ab	128.5 ab	0.92 a	6.35 a	6.20 a	1.03 a	3.10 a
CC at 1500 ppm	114.95 b	124.5 b	0.92 a	6.10 a	6.25 a	0.98 bc	3.04 ab
CC at 2000 ppm	93.80 c	102.3 c	0.92 a	5.65 b	5.70 b	0.99 abc	3.85 a
	2008 Season						
Control	111.55 b	129.8 b	0.86 a	6.50 a	6.10 b	1.07 a	3.63 ab
CC at 500 ppm	134.80 a	151.0 a	0.90 a	5.95 c	6.50 a	0.92 c	2.76 b
CC at 1000 ppm	112.55 b	120.0 b	0.94 a	6.20 b	5.90 bc	1.06 ab	4.75 a
CC at 1500 ppm	113.40 b	116.0 bc	0.98 a	6.10 bc	6.10 b	1.00 b	4.11 ab
CC at 2000 ppm	93.50 c	101.1 c	0.93 a	5.70 d	5.75 c	0.99 b	4.09 ab

Table (2): Effect of foliar application of Choline Chloride (CC) on chemical properties of Peach fruits cv. EarlyGrande during 2007 and 2008 seasons.

Treatments	SSC	TA	SSC/TA	V.C.	Total Sugars	Total Phenols
	2007 Season					
Control	8.50 a	0.70 b	12.14 b	16.70 c	5.35 c	73.2 ab
CC at 500 ppm	8.45 a	0.65 b	13.10 b	15.85 c	5.55 b	72.2 b
CC at 1000 ppm	8.40 a	0.50 c	16.80 a	18.65 b	5.80 a	75.2 a
CC at 1500 ppm	8.40 a	0.60 bc	14.00 b	16.70 c	5.65 ab	74.0 ab
CC at 2000 ppm	8.30 a	0.95 a	8.76 c	22.65 a	5.80 a	74.2 ab
	2008 Season					
Control	7.70 b	0.70 b	11.00 c	15.85 cd	5.70 b	73.4 c
CC at 500 ppm	8.15 b	0.70 b	11.65 bc	16.00 c	5.70 b	76.7 a
CC at 1000 ppm	8.20 b	0.55 c	15.07 ab	17.30 b	6.5 a	76.7 a
CC at 1500 ppm	7.90 b	0.50 c	15.80 a	15.15 d	5.65 b	76.5 a
CC at 2000 ppm	9.15 a	0.95 a	9.66 c	23.40 a	5.75 b	75.4 b

Table (3): Effect of foliar application of Choline Chloride (CC) and storage periods (SP) on Fruit weight loss % and Firmness of Peach fruits cv. EarlyGrande during 2007 and 2008 seasons.

Treatments	Fruit weight loss %					Firmness				
	1 Week	2 Weeks	3 Weeks	4 Weeks	Mean	1 Week	2 Weeks	3 Weeks	4 Weeks	Mean
	2007 Season					2007 Season				
Control	0.00	1.73	3.29	5.83	2.72 BC	5.30	5.20	4.25	3.30	4.51 D
CC at 500 ppm	0.00	2.12	4.31	7.02	3.36 AB	6.40	6.10	5.55	5.30	5.84 AB
CC at 1000 ppm	0.00	1.51	3.10	4.64	2.31 C	6.10	5.45	5.10	4.70	5.34 C
CC at 1500 ppm	0.00	2.57	4.46	6.51	3.39 A	6.55	6.30	5.85	5.10	5.95 A
CC at 2000 ppm	0.00	2.15	4.16	5.58	2.97 ABC	6.45	5.90	5.55	4.90	5.70 B
Mean	0.00 D	2.02 C	3.86 B	5.92 A		6.16 A	5.79 B	5.26 C	4.66 D	
L.S.D. of interaction 5%	1.331					0.354				
	2008 Season					2008 Season				
Control	0.00	1.93	3.70	5.81	2.86 C	6.35	5.45	4.10	3.55	4.86 D
CC at 500 ppm	0.00	1.49	2.79	4.19	2.12 D	6.90	6.55	5.90	5.50	6.21 A
CC at 1000 ppm	0.00	1.76	3.45	4.97	2.54 C	5.65	5.30	5.10	4.70	5.19 C
CC at 1500 ppm	0.00	2.49	4.41	6.24	3.28 D	6.90	6.10	5.55	5.45	6.00 B
CC at 2000 ppm	0.00	2.54	5.03	7.29	3.71 A	7.25	6.45	5.65	5.00	6.09 AB
Mean	0.00 D	2.04 C	3.87 B	5.70 A		6.61 A	5.97 B	5.26 C	4.84 D	
L.S.D. of interaction 5%	0.811					0.358				

Table (4): Effect of foliar application of Choline Chloride (CC) and storage periods (SP) on TSS and TA of Peach fruits cv. Early Grande During 2007 and 2008 seasons.

Treatments	TSS					TA				
	1 Week	2 Weeks	3 Weeks	4 Weeks	Mean	1 Week	2 Weeks	3 Weeks	4 Weeks	Mean
	2007 Season					2007 Season				
Control	8.3	8.1	9.1	9.7	8.80 D	1.05	0.85	0.8	0.75	0.86 A
CC at 500 ppm	8.4	9.5	10.1	10.3	9.58 B	0.85	0.7	0.6	0.575	0.68 BC
CC at 1000 ppm	9.5	10.5	10.7	11.7	10.60 A	0.75	0.63	0.60	0.50	0.62 C
CC at 1500 ppm	7.7	9.3	10.1	10.5	9.40 C	0.95	0.85	0.65	0.58	0.76 B
CC at 2000 ppm	8.2	9.7	9.7	10.4	9.50 BC	0.85	0.80	0.65	0.60	0.73 B
Mean	8.42 D	9.42 C	9.94 B	10.52 A		0.89 A	0.77 B	0.66 C	0.60 C	
L.S.D. of interaction 5%	0.86					0.57				
	2008 Season					2008 Season				
Control	7.5	8.2	8.7	9.5	8.48 E	0.975	0.9	0.75	0.6	0.81 A
CC at 500 ppm	8.0	8.9	9.7	10.3	9.23 D	0.85	0.85	0.75	0.625	0.77 A
CC at 1000 ppm	10.0	10.3	10.7	11.1	10.53 A	0.75	0.63	0.55	0.43	0.59 B
CC at 1500 ppm	8.9	9.7	10.1	10.8	9.88 B	0.95	0.85	0.75	0.55	0.78 A
CC at 2000 ppm	8.9	9.5	9.5	10.2	9.51 C	1.05	0.85	0.75	0.60	0.81 A
Mean	8.66 A	9.32 C	9.73 B	10.38 A		0.92 A	0.82 B	0.71 C	0.56 D	
L.S.D. of interaction 5%	1.00					0.51				

Table (5): Effect of foliar application of Choline Chloride (CC) and storage periods (SP) on TSS/TA and V.C. of Peach fruits cv. EarlyGrande During 2007 and 2008 seasons.

Treatments	TSS/TA					V.C.				
	1 Week	2 Weeks	3 Weeks	4 Weeks	Mean	1 Week	2 Weeks	3 Weeks	4 Weeks	Mean
	2007 Season					2007 Season				
Control	7.92	9.56	11.57	13.00	10.51 C	18.0	13.0	15.0	13.0	14.75C
CC at 500 ppm	9.93	13.57	16.83	18.25	14.65 B	22.0	18.0	18.0	16.0	18.50 A
CC at 1000 ppm	12.73	16.83	17.83	23.40	17.70 A	20.0	20.0	19.0	19.0	19.50 A
CC at 1500 ppm	8.13	10.97	15.64	18.29	13.26 B	15.0	18.0	15.0	15.0	15.75 BC
CC at 2000 ppm	9.67	12.33	15.02	17.33	13.59 B	20.0	16.0	19.0	18.0	18.25 AB
Mean	9.68 D	12.65 C	15.38 B	18.05 A		19.0 A	17.0 AB	17.2 AB	16.2 B	
L.S.D. of interaction 5%	2.92					5.11				
	2008 Season					2008 Season				
Control	7.69	9.11	11.66	15.83	11.08 C	17.0	15.0	15.0	15.0	15.50 B
CC at 500 ppm	9.43	10.51	13.00	16.74	12.42 BC	18.0	15.0	16.0	15.0	16.00 B
CC at 1000 ppm	13.38	16.51	19.63	26.22	18.94 A	21.0	19.0	20.0	18.0	19.50 A
CC at 1500 ppm	9.40	11.44	13.54	19.80	13.55 B	17.5	14.0	15.0	13.0	14.88 B
CC at 2000 ppm	8.48	11.21	12.69	17.00	12.35 BC	18.0	16.0	17.0	19.0	17.50 AB
Mean	9.68 D	11.76 C	14.10 B	19.12 A		18.3 A	15.8 A	16.6 A	16.0 A	
L.S.D. of interaction 5%	2.97					6.91				

Table (6): Effect of foliar application of Choline Chloride (CC) and storage periods (SP) on Total Sugars and Total Phenols of Peach fruits cv. Early Grande during 2007 and 2008 seasons.

Treatments	Total Sugars					Total Phenols				
	1 Week	2 Weeks	3 Weeks	4 Weeks	Mean	1 Week	2 Weeks	3 Weeks	4 Weeks	Mean
	2007 Season					2007 Season				
Control	4.15	4.20	4.65	5.25	4.56 C	76.0	77.5	72.5	72.0	74.50 AB
CC at 500 ppm	4.10	4.65	5.10	5.70	4.89 B	78.5	77.0	77.5	75.0	77.00 A
CC at 1000 ppm	4.80	5.15	5.45	5.60	5.25 A	76.0	74.0	73.0	72.5	73.88 B
CC at 1500 ppm	4.45	4.80	4.80	5.30	4.84 B	75.5	74.5	74.0	72.5	74.13 B
CC at 2000 ppm	4.45	5.25	5.05	5.70	5.11 A	73.5	74.0	74.5	73.5	73.88 B
Mean	4.39 D	4.81 C	5.01 B	5.51 A		75.9 A	75.4 AB	74.3 AB	73.1 B	
L.S.D. of interaction 5%	0.38					5.23				
	2008 Season					2008 Season				
Control	4.25	4.55	4.90	5.45	4.79 C	77.5	76.5	74.5	72.5	75.25 A
CC at 500 ppm	4.45	4.50	4.90	5.05	4.73 C	76.0	74.0	74.0	72.5	74.13 A
CC at 1000 ppm	4.85	5.05	5.30	6.00	5.30 A	76.5	76.0	73.0	70.0	73.88 A
CC at 1500 ppm	4.50	4.90	5.40	5.75	5.14 AB	77.5	73.5	73.0	71.0	73.75 A
CC at 2000 ppm	4.15	4.70	5.25	5.85	4.99 B	75.5	76.0	74.5	73.5	74.38 A
Mean	4.44 D	4.74 C	5.15 B	5.62 A		76.6 A	75.2 AB	73.8 B	71.5 C	
L.S.D. of interaction 5%	0.33					4.53				

CONCLUSION

Fruit weight and SSC/TA ratio were increased by 500, 1000 and 1500 mg/L CC while, fruit acidity was decreased. Sugar, phenol and vitamin C content tended to increase by CC at harvest time. The combination of CC treatments at 1000 and 500 or 1000 mg/L and cold storage at 1ºC resulted in a reduction of weight loss (%). CC in combination with storage resulted in higher fruit firmness, SSC, SSC/acidity and total sugar and a reduction in fruit acidity.

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The Preparation of Paddy Map by Digital Numbers of IRS images and GIS

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Abstract: Preparing updated map of paddy is an important map in the management and region agricultural planning. In this research, surveying of paddy investigated using IRS Satellite images in the Roudbar region, Guilan, Iran. The mean and standard deviation of training and auxiliary pixels of paddy was calculated. Upper and lower limits of DN-olive orchards were distinguished by the adding standard deviation to mean or diminishing of that. After rounding the upper/lower limits of paddy spectrum reflexes, 22-25, 40-98 and 24-136 of spectrum reflexes limits had been considered for bands 1, 2 and 3 with paddy class. In each band, Paddy limits introduced to software and slicing method used to prepare paddy map. Final map of paddy obtained from crossing of these three maps. The paddy map has been crossed by training point map to calculate the accuracy of method. The results indicate that in classification of images with spectrum reflex statistics, more than 73% of training points had again paddy class in the paddy fields classified map.
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Keywords: Species richness; beta-diversity; taxonomic diversity; forest

1. Introduction

As human demands increase, the sustainability of land use is in question. Better land management involves identifying land-use changes, understanding current land-use patterns or features, and assessing economic and ecological benefits and costs that arise from land-use practices, as well as finding the best alternatives for each area (Wu et al., 2001). Remotely sensed data frequently are used to map land surface cover for use in a variety of resource assessment, land management, and modeling applications. Mapping from coarse spatial resolution images and with multispectral instruments necessarily has focused on land cover and broad vegetation types (Loveland, 2000) rather than discrimination of vegetation at a species level.

Rembold et al. (2000) investigated land cover changes in a 22 years period at Lakes region in south of Ethiopia by aerial photographs (1972) and classifying TM land sat images (1994). The analyses indicate that cultivated surface had been increased and more erosion had been occurred in new cultivated lands. Unal et al. (2004) rendered to classify cultivated land and separation of pistachio garden and orchard from the other vegetation in Gaziantep province of Turkish. Ramos et al. (2007) tried to measure and identify of soil movement in various gradient using GPS, GIS and DEM. Also, Moschen et al. (2001) tried to separate agricultural area from non- agricultural area using controlled classification of integrated images of TM5 with IRS IC PAN land sat and ERS 2 radar by maximum likelihood method. In addition to this separation, they tried to separated wheat, maize farm and rangelands.

Using AIF (adaptive image fusion) index, Fletcher (2005) used high resolution QuickBird satellite images to recognize citrus with black mold (capnodium citri) in Texas region of America and indentified it as a suitable method. Das et al. (2009) tried to prepare map for regions with reducing citrus production capacity in Meghalaya region of India using IRS satellite images. The map of regions where citrus production capacity had been reduced was prepared using soil erosion information, vegetation condition and humidity tension. Due to some changes which are created above time in paddy surface preparing updated map of paddy is one of the most important requirements in the management and region agricultural planning. With regard to this that land surveying required high cost and time and also preparing the map through aerial photographs is required to prepare aerial photograph which still along with high cost, use of satellite data along with remote sensing technique may be employed as a useful and effective tool to estimate crop area.

2. Material and Methods

The study area is located between eastern longitudes of 48°55′48″ and 49°52′54″; and northern latitudes of 36°31′19″ and 36°59′57″ that the region area is 4590 km^2. Administrative boundary of the study area includes Roodbar Township along southern portion of Guilan province, Iran. Different image processing techniques are usually available to highlight a certain land use. In present research, a technique that was employed to highlight paddy from other land covers which are going to be described by spectral reflectance stochastic (DN: Digital Number)

of different land covers and slicing. IRS images of July 2006 were used to map olive farming area and software ILWIS 3.3 Academic was used for processing data. Table 1 shows the properties of different bands of IRS images. Field views (189 points) were done to determine accurate positions of land covers including paddy, Olive, hard wood forest, soft wood forest, bare lands, other vegetation, water area and urban regions.

Table 1. Some properties of IRS bands

Band No.	Standard Deviation	Median	Mean
1	13.3	28	19.58
2	51.33	99	67.3
3	47.99	98	67.7

A training and auxiliary points map of different land covers was prepared to overlay on a sample set of color composite (bands 1, 2 and 3). The mean and standard deviation of training and auxiliary pixels of paddy was calculated. Upper and lower limits of DN-paddy were distinguished by the adding standard deviation to mean or diminishing of that ($\bar{X}(B_1, B_2, B_3) \pm 2S.d$). After rounding the upper/lower limits of paddy spectrum reflexes, 22-25, 40-98 and 24-136 of spectrum reflexes limits had been considered for bands 1, 2 and 3 with paddy class. In each band, Paddy limits introduced to software and slicing method used to prepare paddy map. Final map of paddy obtained from crossing of these three maps. The paddy orchards map has been crossed by training point map to calculate the accuracy of method.

3. Results

Table 2 indicates mean, standard deviation and upper/lower limits of training pixels spectrum reflexes-paddy in order to image slicing in bands 1, 2 and 3. As we can see, in band 1, there is a shared DN between Paddy spectrum reflexes and the other surface covers including Olive, other vegetation, hard wood and soft wood forests, and approximately barren land. In band 2 of IRS satellite image, the greatest spectrum reflexes interference with paddy class in vegetation cover, Olive and then soft wood forests in found. In this band, in $\bar{X}(B_1, B_2, B_3) \pm 2S.d$, spectrum reflexes interference of hard wood forest, bare lands and urban regions had been lasted, but DN interference of water zones with paddy is seen. Also, in band 3, there is interference between broad leaf wood, vegetation cover, Olive orchards with paddy, but soft wood DN interference and water zones with paddy had been lusted.

Table 2. Upper and lower limits of spectral reflectance by the mean and standard deviation of spectral reflectance in training and auxiliary points of surface covers

Surface cover \ Band number	$\bar{X} - 2S.d$			$\bar{X} + 2S.d$		
	1	2	3	1	2	3
Paddy	22.4	40.8	97.4	24.9	62.5	135.9
Olive	22.5	51.3	90.0	25.7	77.9	114.7
Other vegetation	23.0	48.7	81.5	26.6	78.7	116.5
Hard wood forest	22.4	31.0	81.4	24.4	39.7	136.6
Soft wood forest	22.4	51.2	48.5	24.4	65.1	61.3
Bare lands	24.8	89.7	74.5	30.2	129.0	105.5
Water area	25.0	57.6	24.8	26.4	67.2	38.0
Urban	23.6	81.0	72.4	32.0	127.5	102.8

* Digital Numbers Mean of Training Points in Olive Orchards Class

** Digital Numbers Standard Deviation of Training Points in Olive Orchards Class

Table 3 indicated the crossing result of training points map with paddy map. According to results, more than 73% of training points in classified paddy map recognized as paddy class. Only 8.5% of training points of soft wood forest class in classified map of paddy by slicing method had paddy class. Also, no one training points soft wood forest were not placed in classified map of paddy class. Also in surface cover classes of barren lands, urban and water, none of the training pixels classified in paddy class. Spectrum reflexes interference of Olive with paddy had been found, so that, 38.8% of training pixels of Olive in the classified map had paddy class. 45.0% of training points in other vegetation were also classified as paddy in classified map.

Table 3. Crossing classified map of olive orchards by training points map

Surface cover	N_t*	N_{t-o}**	N_{t-o}/N_t***
Paddy	1502	1107	73.7
Olive	2016	783	38.8
Other vegetation	293	132	45.0
Hard wood forest	10884	932	8.5
Soft wood forest	208	-	-
Bare lands	38734	-	-
Water area	1134	-	-
Urban	8597	-	-

* Total numbers of training points
** Numbers of paddy class-pixels after crossing classified map of paddy with training points map
*** Paddy class-pixels/total pixels ratio (%)

4. Discussions

The aim of current study was to separate the paddy regions from the other surface areas. In various regions, the type of surface phenomenon impact on map accuracy from classification, intensively. For example, separating of water zones in IRS 3-bands images from surface phenomenon maybe possible, simply which in turn have its own certain condition, so, when the issue of separation one vegetation from the other vegetation is consider the possible of separating is most difficult.

In this research, paddy consider as one class and the other vegetation including orchards, woodlands, garden and etc had been considered in another class by the title of other vegetation. Also, the vegetation of broad-leaf (hard wood) forest and conifer (soft wood) forest each consider in separate classes. With regard to the results, separating paddy helping spectrum reflex statistics cause to separate paddy from forest zones, well. Of course it must consider that, in each of spectrum bands, there is wave interference of some of surface covers, so considering 3 spectrum bands with each other, caused to reduce the interference of reflexes and to increase the possible to separate paddy. Cuneo (2009) provided a map of African Olive distribution was produced from the image analysis and checked for accuracy at 337 random locations using ground observation and comparison with existing vegetation maps. Results indicated that a total area of 1907 ha of dense African Olive infestation was identified, with an omission error of 7.5% and a commission error of 5.4%. Sepulcre-Canto (2009) monitored a total of 1076 olive orchards in area in southern Spain, gathering the field location, field area, tree density, and whether the field was drip irrigated or rainfed by. An approach based on a cumulative index using temperature and the normalized difference vegetation index (NDVI) information for the 6-year ASTER time-series was capable of detecting differences between irrigated and rainfed open-canopy orchards, obtaining 80% success on field-to-field assessments. The method considered that irrigated orchards with equal vegetation cover would yield lower temperature and NDVI than rainfed orchards; an overall accuracy of 75% and a kappa (kappa) of 0.34 was obtained with a supervised classification method using visible, near infrared and temperature information for the 6-year ASTER imagery series.

The results indicated that there would be possible to separate paddy spectrum reflexes from broad leaf forest (hard wood forest), conifer forest (soft wood forest), urban and residential regions, bare lands, ranges and water zones, but because of intensive spectrum reflexes interference, there was not this possible in separate the paddy from other vegetation including the other orchards, woods and parks green spaces.

5. Conclusion

Paddy mapping by spectrum reflections statistic only focusing on paddy spectrum reflexes statistic, the likelihood paddy regions had been separated and preparing the maps is done with regard to goal, that is, paddy and the other surface covers are not consider. As a whole, it seems that, if preparing the map of paddy is doing with the help of spectrum reflexes statistic in the regions with paddy and other vegetation, the separated area must indicated under the title of mixed paddy, olive and other vegetations.

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Survey on some Chemical Pollutant Residues in Catfish at Sharkia Governorate, Egypt

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Abstract: Thirty samples of African catfish (*Clarias gariepinus*) were collected from the Zagazig and Abo Kabeer district markets in Sharkia Governorate for detection and determination of 13 organochlorine pesticides (αBHC, βBHC, γBHC, heptachlor, heptachlor epoxide, aldrin, dieldrin, endrin, γ chlordane, γ endosulfan, pp DDE, pp DDD and pp DDT), 5 organophosphorus pesticides (diazinon, chlorpyrifos, chlorpyrifos methyl, profenophos and disyston) and 11 polychlorinated biphenyls (PCBs) congeners (PCB28, PCB44, PCB70, PCB101, PCB105, PCB138, PCB152, PCB153, PCB180, PCB192, and PCB194). All the tested organochlorine pesticides were detected with the frequency ranged between 30% for αBHC and 76.66% for aldrin + dieldrin. Their mean concentrations varied from 1.9 ppb for aldrin to 122.2 ppb for γBHC. Meanwhile all the tested PCBs were detected except PCB105 with the frequency lies between 10% for PCB28 and 53.3% for PCB152, while; the mean concentrations varied from 3.0 to 89.16 ppb for PCB194 and PCB152 respectively. All the estimated organochlorine pesticides and PCBs were below the permissible limits in all the examined samples. Meanwhile, the tested organiphosphorus compounds were not detected in all the examined samples. The relatively high frequency and levels of organochlorine pesticides and PCBs may be explained by the nature of catfish habits and feeding as exhibited in this study.

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1. Introduction:

The African catfish tend to live in the turbid and cloudy water; furthermore. It is exposed to different types of environmental contaminations rather than another fish types. Because of the relatively high fat contents of the African catfish meat, the fat soluble environmental pollutions as organochlorine pesticides and polychlorinated biphenyls (PCBs) are the more probable pollutant sources of the catfish meat (Holtan, 1998). Furthermore, the organophosphorus pesticides could be polluting the catfish meat via the recent agricultural application.

Pesticides reach aquatic ecosystem by direct application, spray drift, aerial spraying, erosion and runoff from factories and in sewage. Organochlorine pesticides were detected in fresh water fish in previous studies in Egypt (Salah El Dien and Nasr, 2004), the probable sources of this pesticide group originated from previous or illegal using. Organochlorine pesticides cause serious toxic symptoms including developmental abnormalities, growth suppression, disruption of the endocrine system, impairment of immune function, and cancer promotion (El Nemr et al., 2003). On the other aspect, organophosphorus pesticides are regarded as being low persistent compared with organochlorine, but some reports have indicated that residues of organophosphorus are persisting for extended period in organic soil and surrounding drainage systems (Miles et al., 1978).

Polychlorinated biphenyls (PCBs), originally termed "chlorinated diphenyls," were commercially produced as complex mixtures containing multiple isomers at different degrees of chlorination. They entered the environment during their manufacture and use. Today PCBs can still be released into the environment from poorly maintained hazardous waste sites that contain PCBs; illegal or improper dumping of PCB wastes; leaks or releases from electrical transformers containing PCBs; and disposal of PCB-containing consumer products into municipal or other landfills not designed to handle hazardous waste. PCBs may also be released into the environment by the burning of some wastes in municipal and industrial incinerators. Some PCBs congeners elicit a divers spectrum of toxic and biochemical response including body weigh loss, immunotoxicity (Sormo et al, 2009) and induction of gene expression (El Nemr et al., 2003).

Therefore, the objectives of the current study are for detection of organochlorine, organophosphorus pesticides and Polychlorinated biphenyls (PCBs) in catfish samples collected from markets at Sharkia Governorate and comparing the obtained levels with the recommended permissible limits.

2. Materials and methods

Thirty samples of African catfish (*Clarias gariepinus*) were collected from Zagazig and Abo Kabeer district markets in Sharkia Governorate for detection and determination of 13 organochlorine pesticides (αBHC, βBHC, γBHC, heptachlor, heptachlor epoxide, aldrin, dieldrin, endrin, γ chlordane, γ endosulfan, pp DDE, pp DDD and pp DDT), 5 organophosphorus pesticides (diazinon, chlorpyrifos, chlorpyrifos methyl, profenophos and disyston) and 11 polychlorinated biphenyls (PCBs) congeners [PCB28(2,4,4΄), PCB44(2,2΄,3,5΄), PCB70(2,3΄,4΄,5), PCB101 (2,2΄,4,5,5΄), PCB105 (2,3,3΄,4,4΄), PCB138 (2,2΄,3,4,4΄,5), PCB152 (2,2΄,3,5,6,6΄), PCB153 (2,2΄,4,4΄,5, 5΄), PCB180 (2,2΄,3,4,4΄,5,5΄), PCB192 (2,3,3΄,4,5,5΄,6) and PCB194 (2,2΄,3,3΄,4,4΄,5,5΄)].

A. Collection of samples

From each of 30 African catfish samples which collected from the markets, approximately 100 gm of the examined samples (muscles) were taken and placed in polyethylene bags. The samples were identified and kept frozen till the analysis was carried out.

B. Analysis of the organochlorine pesticides and PCBs residues

1- Extraction and preparation of samples

Exactly 20 gm of each examined fish sample was homogenized with 20 gm of anhydrous sodium sulfate with tissue homogenizer till have a fine homogenate. The homogenate was extracted with 100 ml of n-hexane: acetone (2:1) and then the extract was filtered through dry cotton and anhydrous sodium sulphate and evaporated till dryness at 40 ̊C (Amaraneni and Pillala, 2001).

Partitioning technique was performed to remove the dissolved fat from the extract according to Leon et al., (1990).

2-Clean up of samples

Sample extracts applied to chromatography column in 2-3 ml of hexane were eluted successively with hexane florisil (60/100 mesh) activated at 250 °C for 12-15 hours, placed in a desicator until cool, deactivated with 0.5% H_2O, stored in a sealed container in a desicator overnight, and then used within 72h. Columns were rinsed with 100 ml hexane collecting eluant in beaker. Stop the flow before the top of solvent reaches the top of sodium sulfate. Discard the eluant into the waste container. Samples extract were applied to the column in 2-3 hexane, elute the column with 60ml hexane, to elute the organochlorine compounds and polychlorinated biphenyls (PCBs) and collecting eluant in the 100 ml flask and reduce to 0.5 ml (Khaled et al., 2004).

3-Preparation of blank solution

The same volumes of solvents (n-hexane – acetone) and sodium sulfate anhydrous used for fish tissue extraction were subjected to the same extraction, partitioning and clean up procedures as mentioned in the examined samples to detect any possible traces of the studied organochlorine compounds or PCBs in the solvents or distilled water.

4- Quantitative determination of organochlorine pesticides and PCBs

At Pesticide Residue Department, Central Pesticide Laboratory, Hewlett Packard GC Model 6890 equipped with Ni^{63} – electron capture detector. GC conditions: HP- 5MS capillary column (30m length X 0.32mm internal diameter (i.d..), X 0.25µm film thickness), carrier gas: N_2 at a flow rate of 4 ml/min; injector and detector temperatures were 230°C and 300°C respectively. The initial column temperature was initial oven temperature, 180°C for 2 min, raised at 3 °C/min. and then held at 220°C for 1 min., then raised at 9°C /min. to 280°C and then held to 2 minutes, until a total time of 30 minutes had elapsed, DB-17 (J & scientific) capillary column (30m length X 0.32mm initial diameter (i.d.) X25 µm film thickness). Operating temperature were column temperature was programmed 160°C to 230°C at a rate of 3°C /min. to 260°C at a rate 10°C then hold 10 minutes. Injector temperature were 280°C and detector temperature was 300°C with nitrogen carrier gas flow at 4 ml./ min was used to confirm the detected organochlorine pesticides and PCBs.

The organochlorine component and PCBs residue were identified by comparing their retention times with those of the standards quantified by extrapolation of corresponding sample peak areas with those from standard curves prepared for each organochlorine standard and PCBs. Small variations in retention times and response factors of each compound during the experiments were corrected for by obtaining fresh chromatograms of the standard mixture after every nine injections. Standard solutions of concentrations ranging from 0.01 to 0.04

ppm were prepared for each organochlorine and PCBs standard and 1µl was injected into the GC. Peak areas of standard solutions were plotted against their concentrations. A line of best fit was drawn through the point and the limits of detection were taken at 5 times the detector noise level.

5- Determination of percentage rate of recovery

The reliability of the analytical method was examined by fortifying the tested samples with known quantities of tested organochlorine pesticides and PCBs, following the same procedures of extraction, partitioning, clean up and analysis. The percentage rate of recovery of the organochlorine pesticides varied from 65.20% to 97.50% for p-p DDT and γ BHC respectively, while; in PCBs it ranged from 84.65% to 99.98% for PCB28 and PCB138 respectively.

C. Analysis of the organophosphorus pesticides residues

1- Extraction and preparation of samples

Extraction of each tissue sample was conducted as described by Abd El- Kader (1989). Grinded and weighted tissue sample (10 gm.) was placed in high speed blender jar. Then 80 ml n-hexane – acetone (1:1) and 2 gm sodium anhydrous sulphate were added to each sample. The sample and solvent solution were blended for ten minutes, and the extract was washed several times with distilled water in separatory funnel. The sample moisture was dried with anhydrous sodium sulphate and evaporated at 40 ºC in rotary evaporator till complete dryness.

Partitioning technique performed according to Leon et al., (1990) to remove the dissolved fat from the extract. On the other aspect, clean up of the extract was carried out according to Mills et al., (1972).

2- Preparation of blank solution

Exactly 80 ml n-hexane – acetone (1:1) solution and 2 gm sodium anhydrous sulphate was subjected to the same extraction, partitioning and clean up procedures as the examined samples to detect any possible traces of the studies pesticides in solvents or distilled water

3- Quantitative determination of organophosphorus pesticides.

The gas chromatograph used was a Hewlett Packard GC Model 6890 equipped with a Flame Photometric Detector (FPD) with phosphorus filter. A fused silica capillary (PAS-1701), column containing 14% cyanopropylsyloxane as stationary phase (30m length x 0.32 mm internal diameter (i.d) x 0.25µm film thickness), was used for the separation in the GC. CP-CIL-13CB 14% phenyl 1.86 % dimethylpolysiloxane as stationary phase (50m x 0.53 mm i.d x 1µm film thickness) was used to confirm the detected pesticides. GC operating conditions were as the following : Injector and detector temperatures were 240 °C and 250 °C; initial oven temperature, 170 °C for 2 min, raised at 7 °C /min. then held at 230 °C for 2 min., and raised at 10 °C /min. to 240^{o}C and then held to 2 minutes. The carrier gas was nitrogen at 3 ml/min. and hydrogen and air were used for the combustion at 75 and 100 ml/min, respectively.

4- Determination of percentage rate of recovery

As mentioned above in organochlorine pesticides and PCBs, the reliability of the analytical method of the organophosphorus pesticides was examined by fortifying the tested samples with known quantities of tested following the same procedures of extraction, partitioning, clean up and analysis. The percentage rate of recovery of organophosphoru ranged between 78.55% to 95.66% for chlorpyrifos and diazinon respectively.

D. Statistical analysis

The statistical analysis of data was conducted using "Statistic for Animal and Veterinary Science" (Petric and Watson, 1999).

3. Results and Discussion:

As shown in table (1), it revealed that the mean values of αBHC, βBHC and γBHC were 24.56 ±10.85, 2.866 ±0.925 and 122.2 ±28.40 ppb respectively, the levels of αBHC nearly coincided with those recorded by Khaled et al., (2004) in mussels in Egypt (3- 47ppb), also, our estimations of βBHC agreed with those recorded in crayfish (1.16ppb) by Salah El Dien and Nasr, (2004) in Egypt and in Nigerian fish (1.2- 4.9ppb) by Adeyemi et al., (2008). On the other aspect, the estimated values of γBHC in the current study were higher than those detected by Skarphedinsdottir et al., (2010) in fish in Iceland and Moon et al., (2009) in sea foods in Korea. The mean levels of heptachlor and heptachlor epoxide in the present investigation were 35.83 ±9.217 and 2.466 ±0.892 ppb respectively, the detected heptachlor levels were obviously higher than those estimated by Khaled et al., (2004) and Salah El Dien and Nasr, (2004), while; Zidan et al., (2002) estimated higher heptachlor values (16- 957 ppb) in *Clarias Lazara* in Egypt than our estimations. Meanwhile, Salah El Dien and Nasr, (2004) recorded heptachlor epoxide (2.5 ppb) in levels nearly similar with those in the present study. Concerning aldrin and dieldrin, their mean values were 1.90 ±0.605 and

42.30 ±11.68 ppb respectively. The aforementioned levels were parallel respectively with those recorded in fresh water crayfish (Salah El Dien and Nasr, 2004), and in Red sea mussels (Khaled et al., 2004) in Egypt. Meanwhile, Kasozi et al., (2006) in fresh water fish in Uganda detected lower dieldrin levels (0.3ppb) than those recorded in the present study. On the other hand, the mean values of γchlordane residues was 40.733 ±14.459 ppb, this level was obviously higher than those detected in crayfish (1.8 ppb) by Salah El Dien and Nasr,(2004). Moreover, γchlordane residues could not be detected by Salem (2003) in *Clarias Lazara* fish in Upper Egypt. The mean concentration of γendosulfan residues was 19.233 ±4.411 ppb, which was higher than the mean levels (1.7 ppb) obtained in Nile Tilapia in Victoria Lake, Uganda (Kasozi et al., 2006). Concerning endrin levels, its mean concentration in the current study was 30.56 ±5.868 ppb, the other Egyptian study (Salem 2003) recorded lower endrin levels than our estimations. On the other aspect, the mean residue levels of ppDDE, ppDDD and ppDDT in the present study were 5.70 ±1.693, 26.50 ±6.266 and 24.33 ±8.213 ppb, respectively. These levels were higher than those obtained by Abbasy et al., (2003) and Khaled et al., (2004) in *Clarias Lazara* fish and mussels in Egypt, respectively, also; other recent study in USA estimated lower DDT values than our figures in catfish fillet (Schecter et al., 2010). On contrast, Salem (2003) found clearly higher DDT levels (527, 73 and 45 ppb for ppDDE, ppDDD and ppDDT, respectively) in *Clarias Lazara* muscles in Upper Egypt. Furthermore, Storelli and Perrone,(2010) detected higher levels of the total DDT residues (224- 799 ppb) in deep sea fish liver from Mediterranean Sea in Italy.

Table1. Concentrations (ppb) of the organochlorine pesticide residues in the examined catfish samples (n = 30).

Organochlorine Pesticides	Range	Mean*	±S.E.
αBHC	N.D.- 190	24.56	10.85
βBHC	N.D.- 20	2.866	0.925
γBHC	N.D.- 480	122.2	28.40
Heptachlor	N.D.- 180	35.83	9.217
Heptachlor epoxide	N.D.- 20	2.466	0.892
Aldrin	N.D.- 10	1.90	0.605
Dieldrin	N.D.- 250	42.30	11.68
γ Chlordane	N.D.- 270	40.733	14.459
γ Endosulfan	N.D.- 80	19.233	4.411
Endrin	N.D.- 90	30.56	5.868
pp DDE	N.D.- 40	5.70	1.693
pp DDD	N.D.- 110	26.50	6.266
pp DDT	N.D.- 190	24.33	8.213

*: In the mean ±S.E calculation, non detected organochlorine pesticides were considered zero.

Regarding the frequency distribution of the estimated organochlorine pesticide residues in the examined samples, Table (2) showed that all the tested organochlorine were detected in the examined samples and their frequency were 9 (30%), 11(36.66%), 22 (73.33%), 20(66.66%), 23(76.66%),18 (60%), 18 (60%), 19 (63.3%) and 22 (73.33%) for αBHC, βBHC, γ BHC, heptachlor+ heptachlor epoxide, aldrin+ dieldrin, γ chlordane, γ endosulfan, endrin and total DDT respectively. Moreover, Table (2) exhibited that all the estimated organochlorine residues were within the permissible limits in all the examined samples. Meanwhile, the obtained results had higher incidence of organochlorine pesticides than those previously recorded in Egypt by Salem (2003) in *Clarias Lazara* fish.

From the obtained results we can be concluded that, although all the detected organochlorine compounds were within the recommended permissible limits, it detected in the relatively higher frequency and levels comparing with the most previous studies. These results were expected because the examined African catfish

(*Clarias gariepinus*) were caught and sold in agriculture environment (Sharkia Governorate, Egypt) suffered from previous using of the organochlorine pesticides. In spite of prohibiting of the organochlorine pesticides in Egypt since 1980s, the long persistence of these compounds and their fat solubility (Casarett and Doull, 2001) in addition to the nature of catfish habitat which lives in cloudy water and preys on another fish, worm and insects (About catfish, 2009) as previously mentioned are satisfied reasons to explain these relative high frequency and concentrations of organochlorine pesticide residues.

Reversing of the organochlorine results, all the tested organophosphorus pesticides were not detected in all the examined catfish samples.

Table (2). Frequency distribution of the organochlorine pesticide residues in the examined catfish compared with the recommended permissible limits(P.L.) (n=30).

Organochlorine Pesticides	Permissible Limits (ppb)	Not Detected		Within P.L.		Exceeded P.L.	
		No.	%	No.	%	No.	%
αBHC	200[1]	21	70	9	30	0.0	0.0
βBHC	200[1]	19	63.33	11	36.66	0.0	0.0
γBHC	500[2]	8	26.66	22	73.33	0.0	0.0
Heptachlor+ Heptachlor epoxide	300[3]	10	33.33	20	66.66	0.0	0.0
Aldrin+ Dieldrin	300[3]	7	23.33	23	76.66	0.0	0.0
γChlordane	300[3]	12	40	18	60	0.0	0.0
γEndosulfan	100[2]	12	40	18	60	0.0	0.0
Endrin	100[4]	11	36.66	19	63.33	0.0	0.0
Total DDT	5000[3]	8	26.66	22	73.33	0.0	0.0

(1): U.S.F.D.A. (1983) (2): German Food Law (1997). (3): U.S.F.D.A.: (2000).
(4): Codex Alimentarius Commission (2009).

Table (3). Frequency and concentrations (ppb) of polychlorinated biphenyl (PCBs) residues in the examined catfish samples (n = 30).

PCBs Congeners	Positive Samples		Range	Mean*	±S.E.
	No.	%			
PCB28	3	10	N.D. - 260	13.17	9.07
PCB44	4	13.3	N.D. – 70	5.50	2.88
PCB70	8	26.6	N.D. - 210	21.00	9.06
PCB101	9	30	N.D. - 240	34.33	11.68
PCB138	8	26.3	N.D. - 130	14.53	5.39
PCB152	16	53.3	N.D. - 380	89.17	20.57
PCB153	7	23.3	N.D. - 750	53.33	27.65
PCB180	8	26.6	N.D. - 180	27.33	9.53
PCB192	4	13.3	N.D. - 60	6.00	3.0
PCB194	4	13.3	N.D. - 50	3.03	1.85
Total PCBs	22	73.3	N.D. - 1050	267.16	49.82

*: In the mean ±S.E calculation, non detected polychlorinated biphenyls were considered zero.
N.B.: All the examined samples were below the permissible limit of total PCBs in fish (2000 ppb) recommended by U.S.F.D.A. (2007).

As shown in table (3), the mean concentrations of polychlorinated biphenyls (PCBs) in the examined samples were 13.17 ±9.07, 5.5 ±2.88, 21.0 ±9.06, 34.33 ±11.68, 14.53 ±5.39, 89.17 ±20.57, 53.33 ±27.65, 27.33 ±9.53, 6.00 ±3.0, 3.03 ±1.852 and 267.16 ±49.82 ppb for PCB28, PCB44, PCB70, PCB101, PCB138, PCB152, PCB153, PCB180, PCB192, PCB194 and total PCBs, respectively. PCB105 was not detected in all the examined samples. The obtained PCBs levels were coincided with concentrations of total PCBs estimated in marine fish in Iceland (111- 377 ppb) by Skarphedinsdottir et al., (2010). On contrast, Storelli and Perrone,(2010) detected higher mean levels of PCBs (561- 1086 ppb) than those in this study in deep sea fish liver from Mediterranean Sea, Italy. Meanwhile, the most available recent investigations as those by Cirello et al., (2009), Montory et al., (2010) and Boscher et al., (2010) recorded lower PCBs levels than our estimations in the examined fish samples in Italy, Chile and Luxembourg respectively.

As shown in table (3), the estimated PCB28, PCB44, PCB70, PCB101, PCB138, PCB152, PCB153, PCB180, PCB192, PCB194 and total PCBs were detected in 3 (10%), 4 (13.3%), 8 (26.6%), 9 (30%), 8 (26.3%), 16 (53.3%), 7 (23.3%), 8 (26.6%), 4 (13.3%), 4 (13.3%) and 22 (73.3%) respectively. Moreover, all the detected PCBs were below the permissible limits (2000 ppb) recommended by U.S.F.D.A. (2007) in all the examined samples. On the other hand, the number of the detected PCBs among the tested congeners and the frequency of the each detected PCB among the examined samples were higher in the present study than those obtained in the edible fish species in Brazil by Da Silva et al., (2003). Meanwhile Khaled et al., (2004) detected high frequency of PCBs - similar with our figures - within the mussel samples collected from Red Sea, Egypt, although they estimated lower PCBs levels than those in this study.

As the obtained results of the organochlorine compounds, the examined African catfish (*Clarias gariepinus*) had high frequency and considerable levels of PCBs, this result may be attributed to the lipophilic nature of PCBs in addition the high fat contents of catfish meat. Moreover, many of the sold catfish in rural markets caught from the drain water channels which may carried the industrial effluents contained PCBs, especially; this examined fish type grow abundantly in the drain water because of their previously described habits.

Generally, the results of the current study indicated considerable levels of both organochlorine pesticides and PCBs, which were prohibited since a long period. Meanwhile, the permitted organophosphorus compounds were not detected. This result declared the serious degree of organochlorine and PCBs compounds, and exhibited the importance of their banning. Moreover, the present investigation revealed that all the examined samples were fit for the human consumption regarding the estimated chemical pollutants, although their relative slightly high levels. The detected organochlorine and PCBs were not indicate to the illegal use or environmental pollution in the Egyptian ecosystem, other recent studies in more advanced countries as Iceland (Skarphedinsdottir et al., 2010) and Italy (Storelli and Perrone, 2010) recorded high levels of the tested chemical pollutants in the examined fish. We could be concluded that the continuous censorship on the catfish fishing from the drainage channels, continuous monitoring of the chemical pollutant residues in the marketed fish and more scientific attention about catfish feeding and breeding were highly recommended.

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Surface Morphology of the Tongue of the Hoopoe (*Upupa Epops*)

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Abstract: The tongue of birds fills the oral cavity and has a beak- like shape. The hoopoe's beak is long, slender and slightly down curved, however, the hoopoe's tongue is reduced in the buccal cavity. Several studies have shown morphological differences among the tongue of bird species. The aims of this study was to examine the dorsal lingual surface of hoopoe's tongue using scanning electron microscopy and to compare the present results with those reported in other avian species. The Hoopoe's tongue occupy 2/3 length of the beak. The morphological features observed in the lingual surface are follows; the epithelium of the apex is thickly keratinized, large conical papillae are located at the border between lingual apex and body, small conical papillae are located between lingual body and root and numerous lingual glands are located in the anterior part of the lingual body and in the clefts of the lingual root. The observations of the three dimensional structure of the subepithetial connective tissue revealed the presence of a system of laminae or smaller interconnected ridges, depending on the area of the tongue. We have indicated the possibility that the differences in the structures of the avian tongue related to the differences in the feeding habits.

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Keywords: tongue, birds, hoopoe, scanning electron microscopy

1. Introduction:

Morphological studies on the structure of the tongue in birds have been conducted in various species, such as chickens, parrot, geese, eagle, cormorant, owl, peregrine falcon, common kestrel and oriental scops owl (Homberger and Brush, 1986; Iwasaki and Kobayashi, 1986; Iwasaki *et al*., 1997; Jackowiak and Godynicki, 2005; Jackowiak *et al*., 2006; Emura and Chen, 2008; Emura *et al*., 2008; Emura *et al*., 2009).

The previous studies indicates that the avian tongue is a triangular organ, which is filled in the whole lower part of the bill, and is divided into the apex, the body and the root. Morphological and functional studies of various avian species indicated a close correlation of the lingual form, the histological structure of the lingual epithelium and the lingual skeletal apparatus with their feeding habits (Campbell and Lack, 1985; McLelland, 1990; Vollmerhaus and Sinowatz, 1992; Koening and Liebig, 2001; Emura *et al*., 2008, 2009).

This study has carried out to clarify the relationship between the morphological features of the tongue by scanning electron microscopy and the life style in the hoopoe, and it was compared with the results reported by Homberger and Brush (1986) on the African grey parrot, studies by Kobayashi *et al*. (1998) describing the morphology of the tongue in penguins and a study by Jackowiak and Godynicki (2005) on the white tailed eagle.

2. Materials and methods

The tongues of the hoopoe (*Upupa epops*) of the family upupidae (Hoopoes) were used in this study. The tongues were fixed in 10% formalin, post fixed with 1% osmium tetroxide in 0.1M sodium cacodylate buffer at pH 7.2 for 1h at 4°C. Thereafter, the specimens were dehydrated through graded series of ethanol and critical point dried. To show the three dimensional connective tissue structure of the lamina propria of the mucosa, some samples were washed in distilled water after fixation and macerated in 10% NaOH at room temperature for 4 days. After maceration tissues were washed in several changes of the distilled water and post fixed in 1% buffered osmium tetroxide for 1h at 4°C and once again washed three times in distilled water, dehydrated in a series of ethanol and critical point dried. All specimens were mounted on aluminum stubs covered with carbon tabs, and then were sputtered with gold and observed under scanning electron microscopy (JSM-5300) at an accelerating voltage of 15kV.

3. Results

The tongue of the hoopoe is about 2 cm long and occupy 2/ 3 length of the beak. The tip of the tongue is pointed. Three parts are distinguished in the

dorsal surface of the tongue: the apex, the body and the root of the tongue (Fig.1).

The surface of the lingual apex is rough and has a thickly keratinized epithelium (Figs. 2a, b). Large conical papillae are located at the rear end of the lingual apex in the form of letter "w" (Fig.2c,d). The body of the tongue has less keratinized epithelium than the apex. The apices of the lingual papillae are pointed towards the posterior part of the tongue. (Figs. 2c, d). There are numerous orifices of the lingual glands at the anterior border of the lingual body (Fig. 2e). The surface of the lingual body was smooth than that of the lingual apex (Fig. 2f, g).

The border between the body and root is clearly distinguished, and small and large conical papillae are located in this border area (Figs. 3a-c). Large number of wide opening of the lingual glands, which are included in many clefts, in the lingual root, and the number of the openings of the lingual glands in the root is larger than that in the anterior part of the tongue (Figs. 3c-g).

Morphological features of the connective tissue structure of subepithelial papillae was exposed after the removal of the epithelium (Figs.2b, d, g; Figs.3d, e, g). The SEM images of the connective tissue of tissue after macerations indicate that the subepithelial papillae are in fact laminae and ridges of varying height and shape. Fig.2b presents a three dimensional structure of the lamina propria of the lingual apex. The fibers of the connective tissue form thin parallel laminae. On the surface of the conical papillae mucosa, a pattern of connective tissue processes directed backward are located (Fig.2d). In the posterior part of the lingual body, the connective tissue ridges increase and join with each other (Fig.2g). The structure of the lamina propria of the mucosa on the surface of the lingual root is presented in Figs.3d,e,g. Several processes are located and directed backward (Fig.3d). The lingual clefts are deep and contain numerous lingual glands (Fig. 3e). Connective tissue ridges are arranged circularly, forming sheaths around the orifices of the lingual glands (Fig.3g).

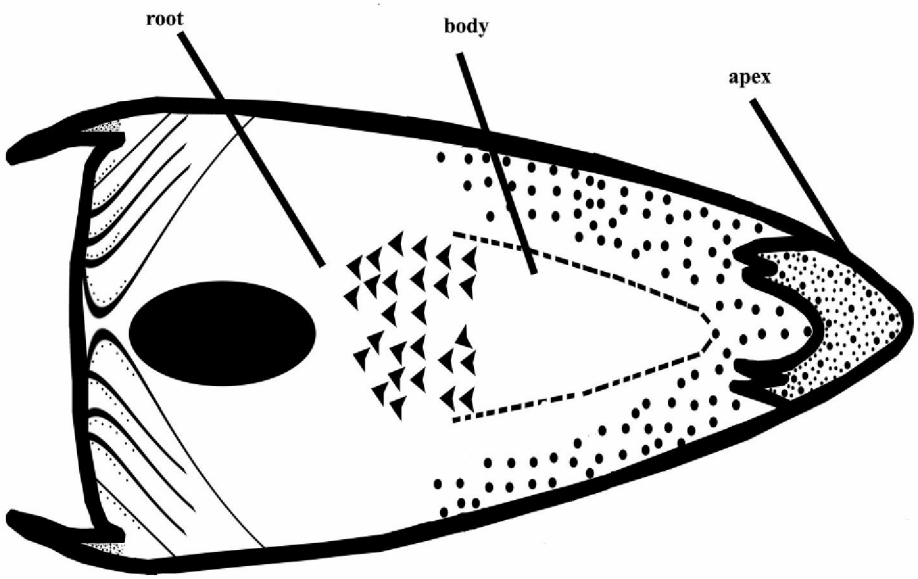

Fig. 1 Diagram showing macroscopic view from dorsal side of the Hoopoe (*Upupa epops*) tongue.

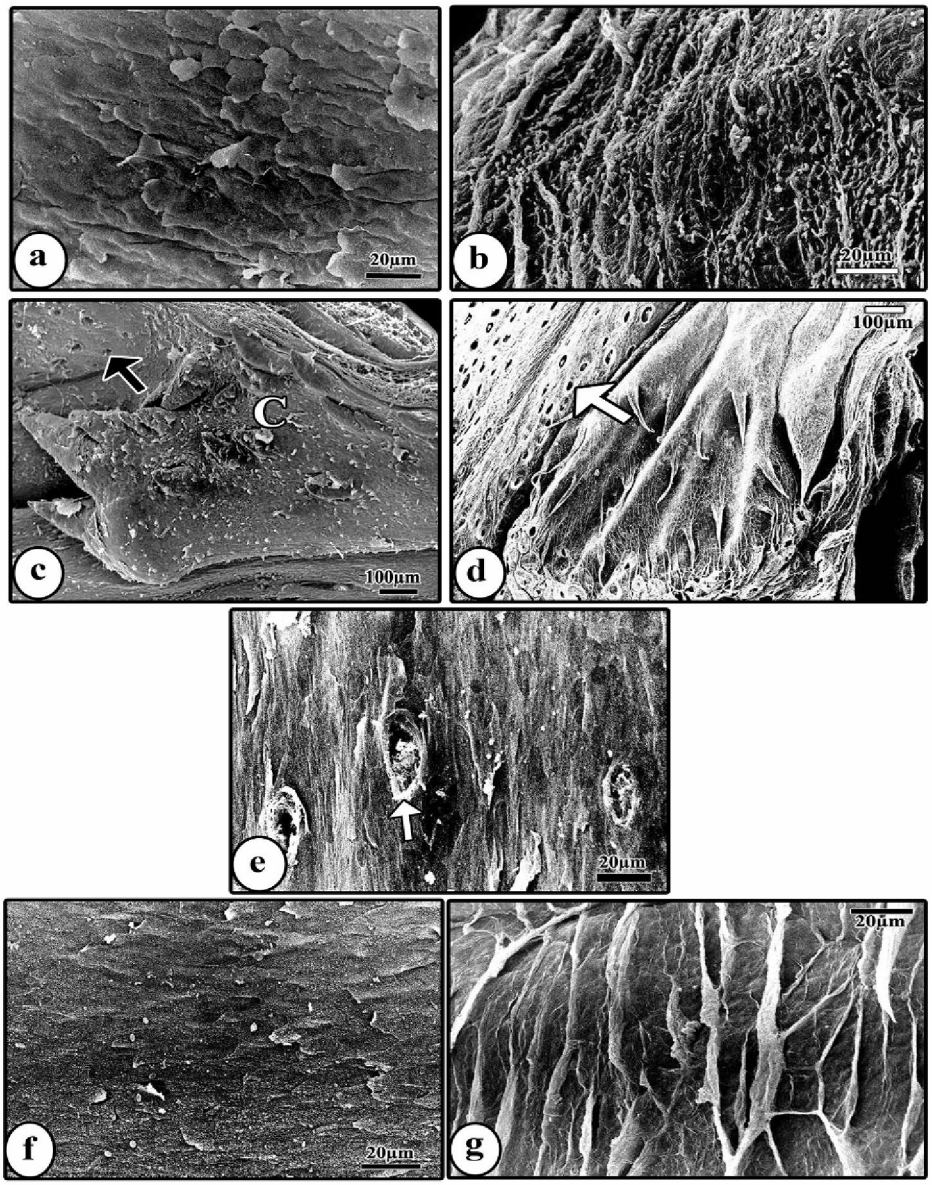

Fig.2 Scanning electron micrograph of the lingual surface apex (a,b) and body (c-g) of the Hoopoe *(Upupa epops)*.

a) the rough epithelium in the lingual apex.

b) surface of the lamina propria of the mucosa after NaoH maceration showing the connective tissue core of the epithelium of the lingual apex.

c) many openings of the lingual glands(arrow) exist in the lingual body beside conical papillae (c).

d) surface of the conical papillae and lingual body with lingual glands (arrow) after NaoH maceration.

e) arrow: glands in the lingual body containing mucus secretions.

f) smooth epithelium in the lingual body.

g) connective tissue core of the epithelium of the lingual body after NaoH maceration.

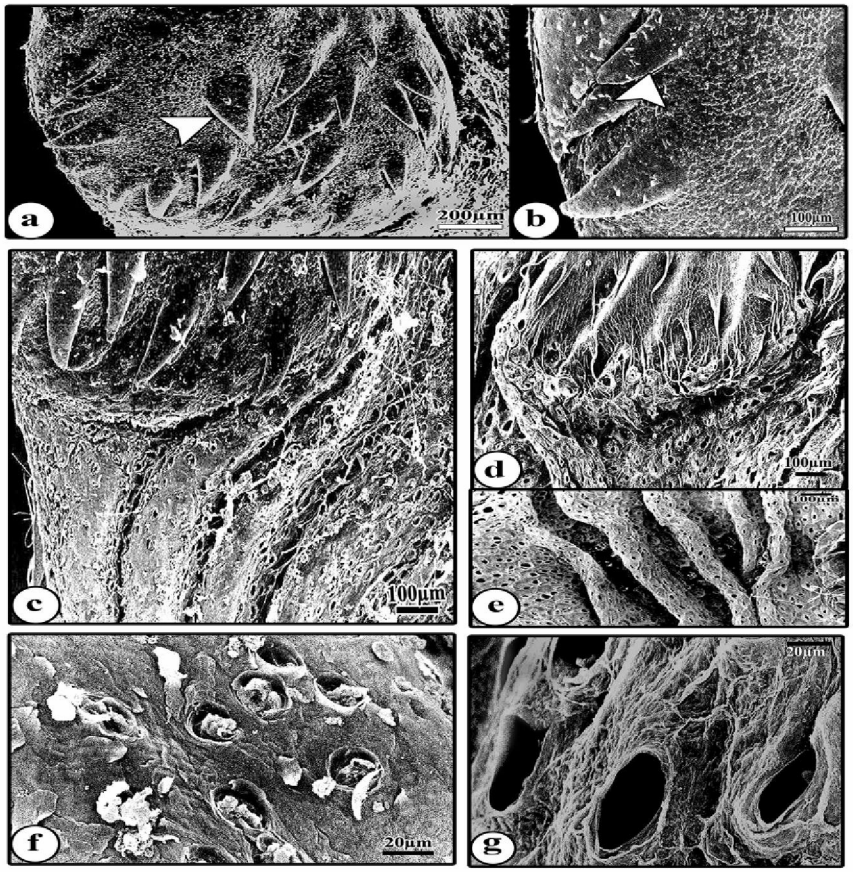

Fig. 3 Scanning electron micrograph of the lingual surface root of the Hoopoe (*Upupa epops*). a, b) conical papillae directed backward at the end of the lingual body. c) epithelium of the lingual root showing many epithelial clefts include numerous lingual glands. d, e) dorsal subepithelium of the lamina propria of lingual mucosa after NaoH maceration. f) many glands exist in the lingual root. g) NaoH macerated sample of the lingual root showing wide orifices of glands.

4. Discussion:

All birds are adapted to their habitats; in the air, on land and on and around fresh water and sea water with respect to food sources. Birds have different feeding habits, with corresponding differences in the structure of their bills and tongues. The structure of the tongue of birds frequently gives some clue to the principal diet and manner of feeding in each species, for example probe or spear in woodpeckers, sieve in ducks, capillary tube in sunbirds, brush in Trichglossidae, rasp in vulture and barbet in penguin.

Hoopoes hunt for prey primarily on the ground in short grasses and on bare soil by walking short distances, stopping Hoopoes eat mainly insects. Hoopoes usually search for the prey on the ground, but may sometimes make a short flight to catch their prey to insert its long slender bill into the ground with the hope of finding food, and then walking off in a different direction. They sometimes probe under and between bark on trees; and other times dig small holes and turn over leaf litter, dry animal droppings, and other material on the ground in search of prey. Hoopoes also make short flights in the air to catch prey. The feeding behaviour of hoopoes is peculiar. It captures its insect food by "gaping". During this operation the bill is first kept closed, then it is driven into the ground. Later the bill is opened against the resistance of earth and the insect food is captured (Kristin, 2001).

In the marginal region between the anterior and posterior parts of the tongue of the chicken, a close array of giant conical papillae was observed, arranged transversely in a row (Iwasaki and Kobayashi, 1986). On the tongue of the goose, giant conical papillae were located in a transverse row between the lingual body and the lingual radix (Iwasaki *et al*., 1997). Large conical papillae, the apices of which were pointed towards the posterior part of the tongue, were located between the body and the root at approximately two thirds of the tongue of the white tailed eagle (Jackowiak and Godynicki, 2005). In the dorsal surface of the hoopoe's tongue, a large conical papillae are found at the posterior border of the lingual apex, and small conical papillae are found between the body and the root of the tongue. The presence of papillae in this region facilitates pushing food towards the posterior region of the conical papillae in which the lingual glands are located.

The main element of the mucosal connective tissue in the lingual body are regular high laminae, arranged parallel to one another. These single or sporadically ramified collagen laminae undoubtedly contribute to the increased area of attachment of the stratified parakeratinized epithelium. Moreover, they could make considerably expanding the boundary surface between the connective tissue and the epithelium for the nutrient exchange between the subepithelial capillary rete and the cells of the desquamate epithelium, which undergoes very intensive renewal. Such an organization of the mucosa may be related to the forces acting on the tongue during the passage of food to the esophagus (Jackowiak and Godynicki, 2005).

In the areas of the tongue covered by the keratinized epithelium connective tissue laminae are low and have the form of ridges. Depending on the segment of the tongue, these ridges are arranged parallel to one another or are joined forming polygonal depressions.

Structures similar to the ridges were observed in the lingual papillae covered by a keratinized epithelium in penguins and chickens (Kobayashi *et al*., 1998; Jackowiak and Godynicki, 2005). The pattern of connective tissue ridges on conical papillae in the penguin resembles parallel striae.

In a few species of birds, it was reported that the anterior and posterior lingual glands were distinguishable based on their location (McLelland, 1975, 1979; Homberger and Meyers, 1989; Vollmerhaus and Sinowatz, 1992). The orifices of the anterior lingual glands are located on the edges of the lingual body or occasionally on the lateral surfaces of the tongue, whereas the orifices of the posterior lingual glands are located on the dorsal surface of the root of the tongue. However, the present study indicated that, in the hoopoe, the anterior lingual glands were located on the entire part of the body and the posterior lingual glands were located on the entire part of the root, and that the number of posterior ones was larger than that of the anterior ones. It postulated that these glands might play a role in lubrication of foods before transporting them to the esophagus. Gargiulo *et al*. (1991) indicated that the secretion of these lingual glands was collected in the subepithelial chamber with the wide orifices, and then was effectively evacuated to the surface of the tongue, and that one of the main components of the secretion was the glutinous mucus which might act as an inhibitors of some bacterial enzymes.

In mammals, some openings of the glandular ducts at the dorsal surfaces of the conical papillae of the lingual radix were observed in the tiger (Emura *et al*., 2004), fox (Jackowiak and Godynicki, 2004) and mole (Jackowiak, 2006). However, the openings of the lingual glands in mammals are a small number than that of the eagle, owl and hoopoe.

The white tailed eagle feeds mostly on fish and the peregrine falcon and common kestrel feeds on small animal. The hoopoe feeds on large insects, their larvae and pupae, and small vertebrates: lizards and geckos. Furthermore, in the white tailed eagle, the crest of the conical papillae found in the lingual body was sites aiding in the transfer of the swallowed food towards the esophagus and at the same time preventing its regurgitatioin (Jackowiak and Godynicki, 2005). In the peregrine falcon and common kestrel (Emura *et al*., 2008), there were observed not only the crest but also the many conical papillae on the lingual body. In hoopoe, large conical papillae are found at the border between apex and body and many conical papillae are found at the posterior part of the body. Therefore, it seems that the differences in the structures of the tongues in the white tailed eagles, peregrine falcons, common kestrels and hoopoes might reflect the differences in their feeding habits.

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Chronic Asthmatic Chest Troubles and Their Effects on Cognitive Functions, Psychosocial Behaviour and Academic Achievment among Children in Egypt

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Abstract: Chronic illness is clearly an important factor affecting psychosocial state of children and adolescents. This case-control study is an effort to clarify the effect of chronic asthmatic chest troubles as a chronic illness on the cognition and psychological aspects of such chronically ill children. This was a case control study conducted at the Chest Clinic of the Abou El-Reesh Children's Hospital, Cairo University. It included 23 children suffering from chronic asthmatic chest troubles (13 boys and 10 girls) with an age range of 6-15 years and a mean age of 9.6±2.67(± SD). Twenty three age and sex matched healthy children and living under the same socioeconomic conditions were taken as controls. The Arabic Version of the Revised Wechsler Intelligence Scale for Children (WISC-R) and Pediatric Symptom Checklist (PSCL) were used to assess the cognitive and psychosocial adjustment among children while the mid-year scores for Mathematics and Arabic language were used to evaluate the academic performance.Our results indicated that chronic asthmatic disease has a negative effect on cognitive abilities, psychosocial behavior and academic achievement of such children.

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1. Introduction:

Health, happiness, independence, and productivity are basic human desires. For children, this means achieving normal growth and development, acquiring a sense of accomplishment, developing an identity, and initiating independence. Although, over time, all children face the same developmental tasks, achieving these developmental milestones depends on many conditioning factors [(1)]. One conditioning factor that greatly influences developmental outcomes and quality of life is chronic illness [(2)].

Chronic diseases affect an estimated 10-20% of all children during childhood and adolescence [(3)]. Chronic chest troubles are the most common cause of chronic illness in children and can affect cognition, psychosocial behavior, and school performance of children. Children with chronic illness are at higher than average risk for behavioral disorders [(4)]. The general consensus of the literature is that chronically ill children are at risk for psychological problems. In chronic childhood conditions, as a whole, the risk of psychopathology is about 2.5 times higher than in the general population [(5)]. One epidemiological study showed that among 4-16 years old child, those with chronic health problems were 2-4 times more likely to have a diagnosable behavioral disorder than their healthy peers [(6)].

In pediatric population psychosocial factors in chronic illness may impede optimal outcome. Overt and covert adjustment problems and psychiatric illness may present as unexplained medical symptoms, non-compliance with medical treatment, school refusal and high-risk behaviors. These signs may alert the physician to the presence of underlying issues in the child and/or the family. Before referral to a mental health professional, the doctor should try to identify the presence of underlying issues, focus on family-centered care and schedule visits to monitor compliance and other issues [(7)].

Early detection and treatment of psychosocial problems may lead to considerable health benefits. Psychosocial problems have a high prevalence rate and lead to high costs of disease. They also cause substantial restrictions in daily functioning in later life and are the major cause of long-term work disability in young adults [(8)]. Only a minority of children with psychological or psychosocial problems are under treatment [(9)]. Research has shown that early detection and treatment improves these children's prognosis substantially, but a complete analysis of its cost effectiveness has yet to be carried out [(10)].

Epidemiological studies showed that roughly one in ten children under the age of 15 suffers from a chronic disease. Other epidemiologic

studies estimated that one third of children less than 18 years of age are suffering from one or more chronic disorders or diseases [11]. In addition, there is an increased prevalence of learning and speech difficulties, sensory dysfunctions, mental handicaps and behavioral problems [12].

The aim of this study was to assess the cognitive functions, psychosocial behavior, and school achievement in chronic asthmatic children and compare them with healthy children of the same age.

2. Subjects & Methods:

This case-control study had been carried out on 46 Egyptian children (23 patients and 23 controls). The two groups were examined and evaluated medically and psychologically, to find whether ill children have more psychological problems than healthy controls. Their age ranged from 6-15 with mean age of 9.6±2.67 years.

The 23 children were previously diagnosed to have chronic asthmatic chest disease and randomly selected. They regularly attended the chest clinic at Abu El- Reesh Children's Hospital, Cairo University. Inclusion criteria included children previously diagnosed to have bronchial asthma, age range between 6-15 years and of both sexes. Exclusion Criteria included children less than 6 years old and those older than 15 years, neurological diseases e.g. cerebral, mentally retarded children and asthmatic chest diseases less than 6 months duration. The control group included 23 healthy children matched for age, sex, educational level and socio-economic state as the patients group. They were selected from the brothers and sisters of the patients group. The controls were free from any chronic illness especially chronic asthmatic chest diseases.

All studied cases were subjected to the following:

History taking: including personal history (age, sex), past history (onset and duration of the disease).

Clinical examination: full clinical examination was done including general examination and local chest examination in order to diagnose chronic chest disease and exclude any other diseases. Diagnosis of chronic chest disease was confirmed by reviewing the laboratory and radiological findings of the patients.

Anthropometric measurements include body weight and height.

Assessment of cognitive abilities: They were assessed by a battery of psychological tests that covered verbal and non-verbal intelligence, memory, learning, problem solving, and attention. The children were individually assessed. All psychological evaluations were administered in one session. The tests used were:

A-The Arabic Version of the Revised Wechsler Intelligence Scale for Children (WISC-R) [13, 14]. This is the most widely used test for intellectual assessment and covers an age range of 6-16years. The test is scored according to a manual from which verbal and performance scores and intelligent quotient are obtained.

B-The Auditory Vigilance Test: It measures the attention ability of the child. It is a measure of the efficiency of identifying signal stimuli in the context from the non-signal ones [15].

C-The Figural Memory Test: This is a measure of the free recall of visual objects [15]. The free recall score is the number of items recalled correctly. The classification score is obtained by counting the number of the shifts from one category to the other, which is made by the subject during his recall. This was considered as an indicator of how he can organize aspects in his memory.

Assessment of psychosocial behaviour: Children's behaviour was evaluated by a brief version of parent-completed Pediatric Symptom Checklist (PSCL-17). Although certain responses may suggest a diagnosis, the PSCL is a screening tool and not a diagnostic one. If positive, the clinician should pursue a brief interview, reviewing the child's major areas of functioning (school, family, activities, friends and mood). If this brief interview supports the PSC findings, the clinician then decides whether a follow-up appointment, further evaluation or referral is indicated [16].

Assessment of Academic Achievement: Was assessed using the mid-year test scores of Arabic language and mathematics subjects for each child. It is considered as a good indicator of academic and learning performance [17]. Each group is classified according to the mid-year scores into good achiever (the mid-year score is ≥ 70%) and poor achiever (the mid-year score is < 70%).

Statistical Methods:

Data were statistically described in terms of range, mean ± standard deviation (± SD), frequencies (number of cases) and percentages when appropriate. Comparison of quantitative variables between the study groups was done using Student *t* test for independent samples. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. A probability value (*p* value) less than 0.05 was considered statistically significant. All statistical calculations were done using the computer programs Microsoft Excel 2003 (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

3. Results

The study included 23 chronic asthmatic patients with an age range of 6-15 years, 10 females (43.5%) and 13 males (56.5%) and 23 age and sex matched healthy controls, Table (1). There was no significant statistical difference between cases and controls as regards age and sex (P > 0.05), on the other hand weight and height were significantly higher in controls compared to asthmatics (P < 0.05).

Table (1) Demographic and anthropometric characteristics of the studied groups

Variable	Asthmatics	Controls	P Value
Age (mean±SD)	9.6±2.67	10.5±2.75	> 0.05
Sex (no, %) Female Male	 10 (43.5%) 13 (56.5%)	 10 (43.5%) 13 (56.5%)	> 0.05
Weight (mean±SD)	25.57±10.65	35.26±7.98	0.008*
Height (mean±SD)	125.32±13.38	135.44±9.03	0.010*

A comparison between cases and controls regarding the cognitive abilities is shown in Table (2). It includes full scale IQ, figural memory test and auditory vigilance test.

(A) Full scale IQ:

The mean full scale IQ for asthmatics was significantly lower than controls (P < 0.05).

(B) Figural Memory test:

(C) Auditory vigilance test:

Test A & B in asthmatics showed significant diminution as regards right answers compared to controls (P=0.000) and wrong answers were significantly higher in asthmatics as compared to controls (P=0.000) as shown in Table (2).

A statistically significant difference was found between cases and controls as regards the free recall and the classification (Fig 1). The mean free recall score for asthmatics was 8.13±3.49 compared to 11.22±2.67 for controls (P < 0.05). For the classification, the mean scores for asthmatics was 2.83±2.19 compared to 4.54±1.59 for controls (P < 0.05).

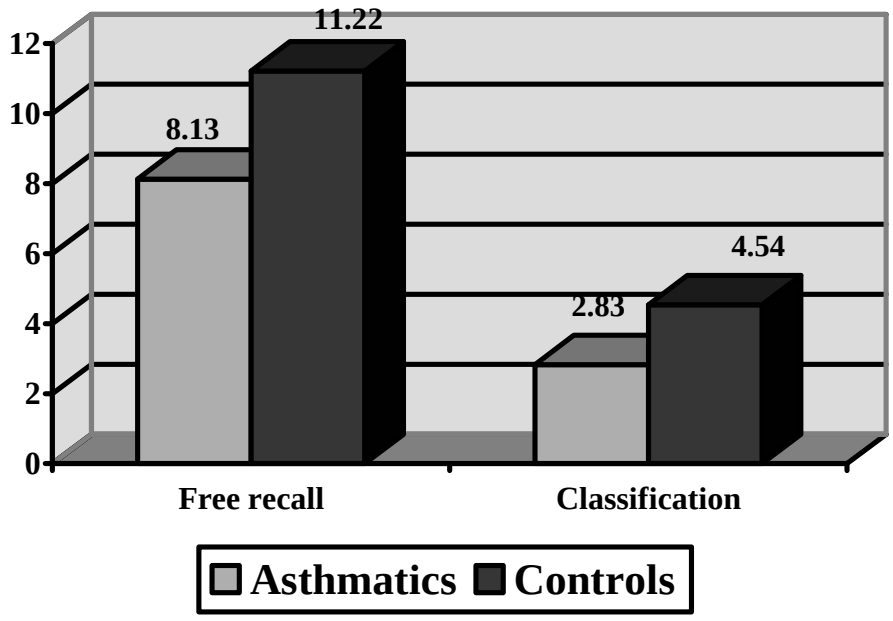

Figure (1) Results of Figural Memory test in asthmatics and controls.

Table (2) Cognitive abilities of the studied groups

	Asthmatics Mean ± SD	Controls Mean ± SD	P Value
(A) Full Scale IQ	73.61±12.79	93.54±12.72	.000*
(B) Figural Memory test			
• Free Recall	8.13±3.49	11.22±2.67	.001*
• Classification	2.83±2.19	4.54±1.59	.034*
(C) Auditory Vigilance test			
Test A			
• Right answers	9.83±2.25	14.00±1.28	.000*
• Wrong answers	3.17±2.25	1.02±1.27	.000*
Test B			
• Right answers	10.65±2.12	12.16±1.24	.001*
• Wrong answers	4.52±2.37	0.88±1.26	.000*

***P < 0.05**

A satatistical difference was seen between asthmatic and control in all domains of psycholohgical behavior while 13% of asthmatic patients show externalizing behavior as well as attention problems, non of the controls (0%) show such behavior (P<0.05). On the other hand 39.1% of the asthmatics show internalizing behavior campared to 6% of controls with a significant statistical difference between them (P<0.05) (Table 3)

Table (3) Results of Psychosocial behavior in asthmatics and controls

	Asthmatics		Controls		Pvalue
	N	%	N	%	
Externalizing behavior					
Positive	3	13	0	0	0.007
Negative	20	87	23	100	
Internalizing behavior					
Positive	9	39.1	1	4.3	0.001
Negative	14	60.9	22	95.7	
Attention problems					
Positive	3	13	1	0	0.007
Negative	20	87	23	100	

Fig 2 showed a comparison between asthmatics and controls concerning academic achievement. It was found that 43.5% of asthmatics show poor mathematics as well as arabic scores significantly difference from control as none of them showed poor scores (0%), (P<0.05).

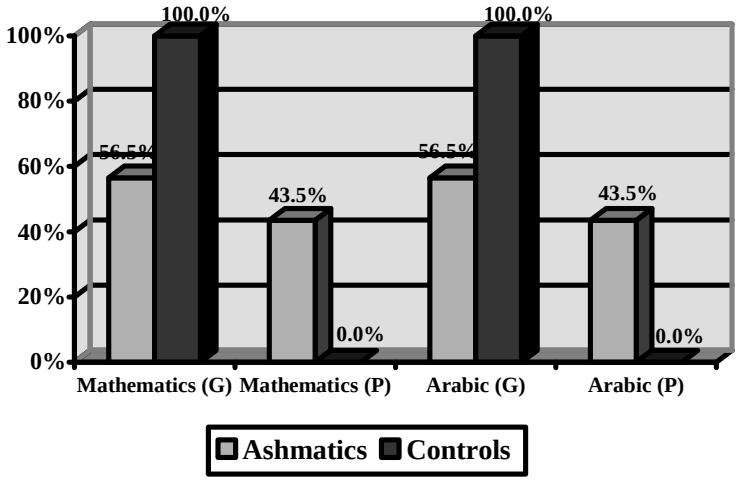

Figure (2) Academic achievement in asthmatics and controls. G: good achievers P: poor achievers

4. Discussion:

Epidemiologic studies indicate that up to half of pediatric visits reflect behavioral, psychosocial, and educational concerns [18]. Most of the pediatrics problems are psychosocial problems that are not severe enough to be classified as psychiatric disorders but interfere with children's social and academic development [19].

Children who have symptoms of illness for more than 3 months, or who require hospitalization or extensive home based services for more than one month in 12 months period are said to have chronic disease [20]. Contrary to El-Baz definition, Leblan et al (2003) [21] suggested that the term chronic illness refers to illnesses that require at least 6 months of continuous medical care, permanent life style changes and continuous behavioral adaptation to the unpredictable course of the illness.

Blckman & Gurka (2007) [22] showed that on top of physical symptoms like coughing, wheezing, and difficulty of breathing, children with asthma are also at increased risk of behavioral, emotional, and developmental problems.

In this study, PSCL was used as a screening test that provided a quick, valid, and reliable method for detection of psychosocial problems. It reflects parent's impressions of their child's psychosocial functioning with acceptable sensitivity and specificity [23]. Using PSCL for behavioral assessment showed that asthmatics had significantly more behavior problems across several domains compared with normal controls.

This study showed significant effect of bronchial asthma on cognitive and behavioral functioning of asthmatic children. These findings are consistent with Naude and Pratorius (2003) [24].

McQuaid et al. (2001) [25], found evidence of behavioral problems in both externalizing and internalizing domains (mainly anxiety and depression). The findings in this study agreed with other studies, which showed a relationship between asthma and internalizing behaviors generally [26].

It was found that in child/adolescent populations with asthma, up to one third met criteria for co-morbid anxiety disorders [27] particularly children with severe asthma [28]. Additionally, a link between higher levels of global internalizing symptoms and childhood asthma has been shown [29]. The present study showed significant differences between asthmatics and controls as regard to attention problems. However, Feldman et al. (2006) [30] didn't find such affection.

Calam et al (2005) [31] documented lower scores on a measure of attention and concentration for children with asthma which is consistent with results of this study. Because behavioral problems are associated with poorer school adjustment and academic achievement, the identification and treatment of problems at this young age potentially could prevent subsequent disruptive behaviors and school difficulties [32]. Agreeing with the results of this study, a research suggested that children with asthma experience more internalizing and total behavior problems than healthy children [33].

Rosa Alati et al (2005) [34] provided information on the association between both asthma prevalence and internalizing symptoms but didn't report any association between externalizing symptoms and prevalence of asthma. Their number of asthmatics was 5153 and they used different methods of assessment. This study showed a greater prevalence of internalizing behavior problems among children and adolescents who had asthma compared with non asthmatics. These results are consistent with the results of Craske et al, (2001) [35]. Consistent with the findings of Gutstadt et al (1989) [36], in this study, academic performance and intelligence test scores indicated that, overall, the academic capabilities of children with asthma were less, compared with healthy children of the same socioeconomic status.

Children with asthma may be at risk for decreased school functioning due to acute exacerbations, increased absenteeism, iatrogenic effects of their asthma medication, and the stress associated with a chronic illness. Factors that may contribute to poor school performance among children with asthma include iatrogenic effects of oral steroids, poor medical management of the disease, and psychological problems [37].

Bender and Bruce (1995) [38] discussed the impact of asthma on children's school achievement. They found negative effect on the educational process, consistent with results of this study. They suggested that the cause may be due to medications that produce mild, temporary changes, affecting learning and classroom performance. For most asthmatic children, their illness does not result in permanent brain function changes that compromise their educational adaptation and performance. Increased school absence, stress of chronic illness, isolation from peers, diminished physical activities, reduced adult expectations and self esteem, and depression can compromise children's academic adaptation and progress.

5. Conclusion and Recomendation

- The present study confirms the effect of chroinc asthma on school age children. It was found that chronic asthmatic children were developmentaly retarded, have more psychological problems, lower cognitive ability, and academic performance compared to healthy controls.

- Medical school training needs to be more focused on the psychosocial issues and psychiatric disorders that affect adolescents with chronic illness rather than on the specific biological issues associated with the medical illness itself.

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Insulin-mimetic activity of vanadium and zinc in diabetic experimental rats

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Abstract: Forty-two adult male albino rats Sprague –Dawley strain were classified into normal control group and five diabetic rat groups which were control (+ve), drug, zinc , vanadium and zinc with vanadium. The diabetic control (+ve) group showed a significant increase in the values of glucose ,glucosalated hemoglobin ,serum alanine and aspartate amino transferase (ALT & AST), alkaline phosphatase (Alk-phos) enzymes, creatinine , urea ,cholesterol, triglyceride (TG), LDL-c, VLDL-c level , cholesterol/ HDL-c ,liver cholesterol, liver total lipid and liver malondialdehyde (MDA) but a significant decrease in final weight, weight gain, FER, insulin, hemoglobin (HB) , packed cell volume ,HDL-c ,liver glycogen, liver glutathione peroxidase (GPX)compared to normal control group. The diabetic rat groups which treated with drug, zinc, vanadium and zinc with vanadium showed a significant decrease in the values of serum glucose , glucosalated hemoglobin, ALT ,AST ,urea, serum cholesterol, triglyceride (TG), LDL-c, VLDL-c level , cholesterol/ HDL-c ,liver cholesterol, liver total lipid and liver malondialdehyde (MDA) but a significant increase in the values of final weight, weight gain percent , FER ,insulin ,packed cell volume (PCV) ,HDL-c ,liver glycogen and glutathione peroxidase (GPX) compared to control (+ve) group.

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1. Introduction

Diabetes mellitus is one of the most widespread diseases in the world and classified as either type 1 insulin-dependent which can be controlled only by daily injections of insulin or type 2 non-insulin dependent which treated by several types of synthetic therapeutics. Diabetes significantly increases risk of developing multiple micro-vascular and cardiovascular complications. The cardiovascular events associated with type 2 diabetes and the high incidence of other macro-vascular complications, such as stroke and amputation (Huang et al., 2007).Diabetes mellitus needs safe and efficient treatment. Because of the failure and secondary effects of promising treatments, many people resort to using alternative therapies for illnesses (Ryan et al., 2001).

Historically, vanadium was used to treat diabetes mellitus dates backed to 1899, before the discovery of insulin in 1921and since then numerous studies have described the in vivo and in vitro anti-diabetic effects of vanadium salts and compounds (Mukherjee et al .,2004). Vanadium with atomic number 23, atomic weight 50.9415 and oxidation states from III to V has a wide variety of biochemical and physiological functions. Among them, an insulin-mimetic antidiabetic effect is the most striking, the effect being provided by the oxidation states of vanadic V (III), vanadyl V (IV) and vanadate V (V) (Tracey and Crans ,1998). Vanadium influences the behavior of enzymes, regulates the activities of second messengers, signal transduction cascades and carbohydrate metabolism, mimics insulin and growth factor activities, stimulates protein tyrosine kinase and inhibits phosphotyrosine phosphatases and modulates gene expression (Srivastava and Mehdi, 2005 and Antonio et al., 2009).

Zinc with atomic number 30, atomic weight 65.39 and oxidation state II is an essential element in all living systems and plays a structural role in many proteins and enzymes. Many proteins have been found to have a zinc-containing motif that serves to bind DNA embedded in their structure (Wolfgang and Harold 2006). Zinc is essential for growth and development. At the cellular level, it is critically involved in proliferation, differentiation, and apoptosis. Zinc requires in immunity, intermediary metabolism, DNA metabolism and repair, reproduction, vision, taste, and cognition/behavior. Zinc was found to have important physiological and pharmaceutical functions involving insulin-mimetic activity (Song et al., 2001 and Yutaka et al., 2004).

The purpose of this study was to investigate the effect of vanadium and zinc or both on diabetic rats.

2- Materials and methods

A – Materials:

1-Chemicals and drugs

Streptozotocin was procured from Sigma, St. Louis, MO, USA. Octozinic capsules produced by October pharma S.A.E and contain 110 zinic sulphate heptahydrate. The therapeutic human dose was 220 mg daily.Vanadyl sulfate-3-hydrate was obtained from Hanawa Extra Pure Reagent China. The human insulin mimics dose of vanadium for treatment of diabetes mellitus was 100 mg daily (Boden et al., 1996). Amaryl drug is antidiabetic drug, produced by Saofi –Avents Egypt under licence of Saofi –Avents Germany. It is antidiabetic sulfonylurea. Each tablet contains 2mg glimepiride. The human therapeutic dose is 4 mg daily. The human therapeutic dose of zinc sulphate heptahydrate , vanadyl sulfate-3-hydrate and amaryl drug were converted to rat dose according to Paget and Barnes, (1964) that were 20 , 9 and o.36 mg/Kg body weight, respectively and dissolved in distilled water and given to rats by oral intubations. Biochemical kits were purchased from Alkan Co. for Chemicals and Biodignostics , Dokki ,Egypt.

2-Experimental animals:

A total of forty-two Sprague –Dawley adult male rats were purchased from the National Research Center, Giza, Egypt. The average weight was 130±8 g.

3- The standard diet:

The standard diet was performed according to NRC (1995) which comprised of casein (200g/kg), corn starch (497g/kg), sucrose (100g/kg), cellulose (30 g/kg), corn oil (50g/kg), mineral mixture (100g/kg), vitamin mixture (20g/kg) and DL-methionine (3g/kg).

B- Methods

1-Experimental design:

Rats were housed in wire cages under the normal laboratory conditions and fed on basal diet for a week as adaptation period. Food and water were provided ad-libitum. The rats were randomly classified into six groups (7 rats each). The first group kept as normal control fed basal diet only. The other five groups were injected with a single intraperitoneal dose of streptozotocin (55 mg/kg body weight) in 0.1 M citrate buffer of pH 4.5 then supplied with 5% glucose solution for 48 h after injection in order to prevent hypoglycemia (Peschke et al .,2000). After four days, blood samples were taken from orbital plexus for estimation of glucose. The rats having persistent hyperglycemia were considered as diabetic rats and used for the experiment. The diabetic rats were classified into control (+ve) and treated four groups that were drug, zinc, vanadium and zinc with vanadium.

The food intake was calculated daily and the body weight gain was recorded weekly. Feed efficiency ratio (FER) was determined by Chapman et al., (1950) as following: FER = weight gain (g)/ feed intake (g).

2-Collection of blood, liver samples and Pancreas:

At the end of experiment (ten weeks), rats were anesthetized, blood sample were collected from hepatic portal vein in clean centrifuge tubes. The liver of sacrificing rats was removed by careful dissection, blotted frees of adhering blood, washed with cold saline solution, and dried between two filter papers. Livers perfuse with 50 to 100 of ice cold 0.9%NaCL solution. Pancreas was dissected out and immediately washed in ice-cold saline and fixed in 10% neutral buffered formaldehyde solution at pH 7.5, cleared in xylol, and embedded in paraffin. 4-5 µm thick section were prepared and stained with Hematoxylin and Eosin (H&E) for subsequent histopathological examination according to (Bancroft et al., 1996).

3- Blood analysis:

First part of blood samples was heparenized for estimation of hemoglobin and packed cell volume (PCV) (Drabkin, 1949 and Mc Inory 1954). Second part of blood was collected in tubes containing potassium oxalate and sodium fluoride for the estimation of glucose by *O*-toluidine method (Sasaki et al., 1972).Third part of blood was left to coagulate then centrifuged at 3000 rpm for 15 minutes to obtain serum. Serum insulin and glucosalated heamoglobin (Hb A1c %) were estimated according to Wilson and Miles (1977) and Abraham et al., (1978).Serum alanine and aspartate aminotransferase (ALT&AST), and alkaline (AP) activity enzymes were estimated according to Reitman and Frankel (1957) and Kind and King (1954), respectively. In addition, creatinine and urea were estimated according to Bonsens and Taussky, (1984), and Patton and Crouch, (1977), respectively.

Serum cholesterol, triglycerides, high density lipoprotein cholesterol, low and very low density lipoprotein cholesterol were determined according to Trinder and Ann (1969), Young and Pestaner, D.L. (1975) ,Richmond (1973) , Fruchart, (1982) and Friedwald, et al.,(1972),respectively. Atherogenic index (cholesterol /HDL-c) was calculated according to Castelli and levitar, (1977).

4- Liver analysis:

Livers samples were analyzed for estimation of glycogen, cholesterol and total lipids according to Rerup and Lundquist, (1967), Abell et al., (1952), and Folch et al., (1957), respectively. Liver glutathione peroxidase (GPX) and malondialdehyde (MDA) were estimated according to Weiss et al., (1980) and Draper and Hadley,(1990), respectively.

8-Statisticl analysis:

Collected data were presented as mean ±SD and statistically analyzed using one way analysis of variance (ANOVA).Student "t" test was used for significance according to Artimage and Berry (1987).

3. Results and Discussion

The diabetic control (+ve) group showed a significant decrease in final weight, weight gain and FER at p< 0.01 compared to normal control group. The diabetic rat groups which treated with drug, zinc, vanadium and zinc with vanadium showed the values of final weight, weight gain percent, food intake and FER around the values of normal control group. The values of final weight, weight gain percent and FER were increased in all treated groups compared to control (+ve) group as shown in table (1).

Nutritionally, vanadium is thought to be a cofactor in various enzymatic reactions. Data from animal and human studies suggest that vanadium mimics the action of insulin.2 .Consequently; it may serve a beneficial role in promoting healthy glucose metabolism in individuals with diabetes. Vanadium deficiency in animals manifests as diminished growth, reproductive impairment, and disruptions of metabolic and cellular function in the kidney, heart and brain. Vanadium has been included in the list of 40 essential micronutrients that are required in small quantities for normal metabolism as well as proper growth and development of mammals (French and Jones, 1993 and Verma et al., 1998). On the other hand, Zn (II) ion, an essential trace element with relatively low toxic profile in animals and humans and has been proven to have a pronounced insulinomimetic activity. Zinc occurs in hundreds of enzymes and in thousands of protein domains. Zinc is essential for growth and development. At the cellular level, it is critically involved in proliferation, differentiation, and apoptosis (Wolfgang and Harold 2006).

The diabetic control (+ve) group showed a significant increase in the values of glucose and glucosalated hemoglobin at p< 0.001& 0.01,respectively and a significant decrease insulin, hemoglobin (HB) and packed cell volume (PCV) at p< 0.001,0.05& 0.01,respectively compared to normal control group. The diabetic rat groups which treated with drug, zinc, vanadium and zinc with vanadium showed a significant increase in the values of glucose (p< 0.05& 0.01) and a significant decrease insulin at p< 0.05compared to normal control group while showed a significant decrease in the values of both glucose and glucosalated hemoglobin and a significant increase in the values of insulin and packed cell volume (PCV) compared to control (+ve) group as shown in table (2).

A beneficial effect of vanadyl sulfate at a dose of 100 mg/day for three weeks appeared in improving insulin sensitivity. Measurement of fasting plasma glucose and insulin-mediated glucose disposal during pre- and post-treatment periods showed a beneficial effect of vanadyl sulfate on improving both hepatic and peripheral insulin sensitivity (Cohen et al., 1995). The reductions in fasting blood glucose and hemoglobin A1c (HbA1c), and improved responses to oral glucose tolerance testing, in vanadium treatment of type 2 diabetes compared to exacerbated diabetic symptoms in the two placebo controls (Thompson et al., 2009). Vanadium increase insulin sensitivity due to inhibit protein tyrosine phosphatase, reduce gluconeogenesis and increase glycogen deposition (Sakurai et al., 2006). The elevation in the glucose levels of STZ-rats treated with Vanadyl complex was significantly lower than those of the control, indicating that vanadyl complex improved the diabetic state of animals. Similarly, zinc (II) complex substantially lowered the blood glucose levels in mice (Akira et al., 2009). The effects of Zn (II) ion on both glucose oxidation and lipolysis stimulation are inhibited by extracellular catalase, largely resulting from H2O2 generation. Zn (II) ion increase of specific insulin binding and stimulates both lipogenesis and glucose transport in the adipocytes. Zn (II) ion was indicated to relate to the carbohydrate metabolism through insulin or insulin receptor (Wolfgang and Harold 2006). Zn2+ is an essential element for normal exocrine and endocrine function of the pancreas. Serum glucose concentration is another parameter associated with liver and pancreatic function. Elevated glucose levels in serum can be associated with a decreased pancreatic endocrine function. Additionally, since glucose is regulated by the liver, elevated serum glucose concentration may also be related to exposure to hepatotoxins (Fugono et al ., 2002 and Liu et al., 2009).

The diabetic control (+ve) group showed a significant increase in the values of serum alanine and aspartate amino transferase (ALT & AST), alkaline phophatase (Alk-phos) enzymes, creatinine and urea at p< 0.001 compared to normal control group. The diabetic rat groups which treated with

drug, zinc and vanadium showed a significant increase in the values of serum amino transferase (ALT & AST), alkaline phophatase (Alk-phos) enzymes, creatinine and urea at p< 0.05 & 0.01 while the diabetic rat group which treated with zinc with vanadium showed a significant increase in the above mentioned parameters except the value of AST compared to normal control group. The diabetic rat groups which treated with drug showed a significant decrease in the values of serum ALT , AST and urea while the diabetic rat group which treated with zinc showed a significant decrease in the values of serum ALT , AST, alkaline phophatase enzymes and urea compared to control (+ve) group. The diabetic rat groups which treated with vanadium or zinc with vanadium showed a significant decrease in the above mentioned parameters compared to control (+ve) group as shown in table (3).

Vanadium possesses a regulatory role in the biological system, influences a number of enzymes, regulates the functions of several second messengers and modulates a battery of genes. The discovery of several pharmacological properties, such as the insulin-mimetic action, antihyperlipidemia, antihypertension, antiobesity, enhancement of oxygen affinity of hemoglobin and myoglobin, and diuretic action, opens up a number of therapeutic avenues of this trace element (Mukherjee et al .,2004 Abd El-Ghanny 2007). ZnCl2 supplementation was reported to increase liver enzyme levels. Serum creatinine is a marker of liver and kidney function. The creatinine precursor, creatine phosphate is biosynthesized in the liver and its muscle byproduct; creatinine is maintained by the kidney. Zinc stabilizes membranes structures and cellular components, is involved in the synthesis of growth hormone, alkaline phosphatase and collagen (Johnson et al., 2010).

The diabetic control (+ve) group showed a significant increase in the values of cholesterol, triglyceride (TG), LDL-c, VLDL-c level and cholesterol/ HDL-c at p< 0.001 but a significant decrease in HDL-c at p<0.001 compared to normal control group. The diabetic rat group which treated with drug showed a significant increase in the values of triglyceride (TG), LDL-c and VLDL-c level at p< 0.05 & 0.01 but a significant decrease in HDL-c at p<0.05 compared to normal control group. The diabetic rat groups which treated with zinc and vanadium showed a significant decrease in HDL-c at p<0.05 but a significant increase in serum LDL-c and VLDL-c at p< 0.01 &0.05, respectively while the diabetic rat group which treated with zinc with vanadium showed a significant increase in serum LDL-c and VLDL-c at p< 0.01 &0.05, respectively compared to normal control group.

The diabetic rat groups which treated with drug, zinc, vanadium and zinc with vanadium showed a significant decrease in the values of cholesterol, triglyceride (TG), LDL-c, VLDL-c level and cholesterol/ HDL-c but a significant increase in HDL-c compared to control (+ve) group as shown in table (4).

Vanadium acts by improving the sensitivity of insulin in type 1 and type 2 diabetes. It has also been shown to reduce cholesterol levels and blood pressure (Beliaeva et al, 2000). Adipocytes play a crucial role in energy storage and homeostasis by converting free fatty acids into triglycerides and by producing a variety of adipokines, and therefore provide a very useful model for addressing questions related to insulin signaling and the interface between sugar and fat metabolism (Andre et al ., 2006). Zn2+ supplementation at high levels increases serum Zn2+ concentration, serum LDL-c, HDL-c, serum total cholesterol and serum triglycerides. Therefore, ZnCl2 supplementation might be beneficial in obesity situation (Roozbeh et al., 2009).

The diabetic control (+ve) group showed a significant increase in the values of liver cholesterol, total lipid and malondialdehyde (MDA) at p< 0.001 but a significant decrease in the values of liver glycogen and liver glutathione peroxidase (GPX) compared to normal control group. The diabetic rat groups which treated with drug and zinc showed a significant increase in the values of liver total lipid and malondialdehyde (MDA) at p<0.05& 0.001, respectively while the diabetic rat groups which treated with vanadium and zinc with vanadium showed a significant increase in the value of liver malondialdehyde (MDA) at p<0.01 compared to normal control group.

The diabetic rat groups which treated with drug, zinc, vanadium and zinc with vanadium showed a significant increase in the values of liver glycogen and glutathione peroxidase (GPX) but a significant decrease in the values of liver cholesterol, liver total lipid and malondialdehyde (MDA) compared to control (+ve) group as shown in table (5).

Vanadium has insulin-like effects and is currently being considered for oral therapy. It also reduces gluconeogenesis and increases glycogen deposition. Vanadium salts induced sustained falls in blood glucose in diabetic rodents (Ray et al. 2004). vanadium inhibits hepatic lipid peroxidation and superoxide dismutase (SOD) and elevation of glutathione (GSH) status as well as cytochrome P450 (CYP) and glutathione S-transferase (GST), indicating modulation of the hepatic antioxidant as well as phase I and II xenobiotic metabolizing

enzymes (Chakraborty et al., 2007 and Anupam et al., 2010). Zn2+ is a major requirement for all life forms and promotes physiological and biochemical functions. Biochemically, Zn2+ is very important in many cellular and biochemical functions such as acting as a cofactor and serving as an integral constituent of many antioxidant enzymes such as superoxide dismutase1and3and several other metalloenzymes (Yutaka et al .,2004 and Franklin et al., 2005). Zinc is an essential component of a great number of zinc dependent enzymes, which participate in antioxidant processes and in the synthesis and degradation of carbohydrates, lipids, proteins and nucleic acids (Koury and Donangelo, 2003).

The histopathological examination showed that control (-ve) rat group showed normal islets with clusters of purple stained β-cells with a normal architecture of pancreas (Pict 1) while the section of control (+ve) rat showed atrophy of β-cells and vacuolar degenerative changes in islets (Pict 2). The diabetic rat group which treated with drug showed normal histological structure (Pict 3). Diabetic rat group which treated with vanadium showed β-cell degeneration (Pict 4) while diabetic rat group which treated with zinc showed reduction in β-cell numbers with increase in islets(Pict 5).The diabetic rat group which treated with zinc with vanadium showed less marked cellular degeneration of pancreatic β-cell(Pict 6).

The obtained results were in parallel with the histopathological examination of the pancreas. Streptozotocin molecules might increase the production of oxygen free radicals that play a crucial role in determining tissue injury as exert direct or indirect effects on islet endothelium and mediate fragmentation of nuclear DNA in beta cells leading to histological changes as well as functional abnormalities (Kakkar et al., 1998). Vanadium can partially preserve β-cells and increase in the number of surviving β-cells through amelioration of hyperglycemia but cannot completely prevent β-cells cytotoxicity from streptozotocin. (Ramachandran et al., 2003)

Table (1): Mean values ± SD of body weight gain, food intake and feed efficiency ratio (FER) of the experimental rat groups.

Variables / Groups	Initial Weight (g)	Final Weight (g)	Weight Gain (g)	Food Intake (g/d)	FER
Control (-ve)	130.56 ± 3.55[a]	210.71± 5.14[a]	61.36± 3.22[a]	18.32± 1.14[a]	0.072± 0.002[a]
Control (+ve)	132.17± 3.45[a]	168.32± 4.13[b**]	27.35± 2.17[b**]	16.88± 1.20[a]	0.035± 0.001[b**]
Drug	135.31± 3.71[a]	211.15± 5.61[a]	56.04± 2.69[a]	18.41± 1.35[a]	0.068± 0.003[a]
Zinc	131.45± 3.25[a]	199.89± 4.98[a]	52.06± 2.61[a]	17.55± 1.12[a]	0.064± 0.001[a]
Vanadium	133.65± 2.99[a]	205.11± 5.47[a]	53.46± 2.68[a]	17.90± 1.31[a]	0.066± 0.004[a]
Zinc + Vanadium	134.40± 2.49[a]	213.61± 5.66[a]	58.93± 3.11[a]	18.35± 1.11[a]	0.071± 0.003[a]

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c) denote significant difference.

Table (2): Mean values ± SD of glucose, insuline, hemoglobin (HB), packed cell volume (PCV) and glucosalated hemoglobin HbA_{IC} of the experimental rat groups.

Variables / Groups	Glucose (mg/dl)	Insulin (µ/l)	HB (g/dl)	PCV %	HbA_{IC} %
Control (-ve)	90.78± 4.31 ^{d}	19.70± 1.51 ^{a}	12.30± 2.11 ^{a}	35.18± 29.71 ^{a}	4.85± 0.58 ^{b}
Control (+ve)	210.77± 6.22 ^{a***}	7.88± 1.11 ^{c***}	9.11± 1.82 ^{bc*}	29.71± 2.14 ^{b**}	7.11± 0.66 ^{a**}
Drug	115.33± 5.17 ^{c*}	16.01± 1.25 ^{b*}	10.88± 1.22 ^{ac}	33.25± 2.99 ^{a}	5.99± 0.85 ^{b}
Zinc	129.41± 6.01 ^{b**}	15.91± 1.25 ^{b*}	11.11± 1.03 ^{a}	34.11± 3.15	5.44± 0.90 ^{b}
Vanadium	130.71± 5.10 ^{b**}	15.41± 1.19 ^{b*}	10.78± 1.12 ^{a}	33.20± 3.11 ^{a}	5.60± 1.01 ^{b}
Zinc + Vanadium	118.35± 5.01 ^{c*}	16.36± 0.99 ^{b*}	11.81± 1.06 ^{ac}	34.51± 2.87 ^{a}	5.01± 0.68 ^{b}

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c) denote significant difference.

Table (3) The Mean values ± SD of serum amino transferase (ALT & AST), alkaline phophatase enzymes (Alk-phos), creatinine and urea of control and diabetic treated rat groups

Variables / Groups	ALT (µ /ml))	AST (µ /ml)	Alk- phos (g/dl)	Creatinine (mg/dl)	Urea (mg/dl)
Control (-ve)	17.15± 1.66^{c}	35.76± 3.11^{c}	30.80± 2.69^{c}	0.30± 0.001^{c}	33.87± 3.50^{c}
Control (+ve)	39.62± 3.45 ^{a***}	59.20± 6.01 ^{a***}	50.61± 5.16 ^{a***}	1.33± 0.03 ^{a***}	60.14± 1.16 ^{a***}
Drug	30.21± 3.21 ^{b**}	48.18± 5.11 ^{b**}	45.01± 5.31 ^{ab**}	1.01± 0.05 ^{ab**}	49.15± 6.11 ^{b**}
Zinc	25.32± 2.63 ^{b*}	40.17± 5.16 ^{b**}	41.29± 4.91 ^{b**}	0.99± 0.4 ^{ab**}	47.13± 5.31 ^{b**}
Vanadium	27.11± 3.61 ^{b*}	43.51± 5.14 ^{b**}	42.60± 4.80 ^{ab**}	0.95± 0.05 ^{b**}	48.22± 5.08 ^{b**}
Zinc + Vanadium	23.14± 3.11 ^{b*}	38.26± 4.11 ^{bc}	37.77± 3.67 ^{b*}	0.65± 0.01 ^{b*}	45.11± 4.86 ^{b**}

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c) denote significant difference.

Table (4) The Mean values ± SD of serum lipid patterns of control and treated rat groups

Variables / Groups	cholesterol (mg/dl)	TG (mg/dl)	HDLc (mg/dl))	LDLc (mg/dl)	VLDLc (mg/dl)	Cholesterol /HDLc
Control (-ve)	91.81± 5.14 ^{b}	43.35± 4.71 ^{c}	37.11± 3.60 ^{a}	46.03± 5.69 ^{c}	8.67± 1.12 ^{c}	2.47± 0.24 ^{b}
Control (+ve)	178.33± 11.34 ^{a***}	70.81± 8.11 ^{a***}	26.31± 2.67 ^{b***}	137.86± 10.33 ^{a***}	14.16± 1.35 ^{a***}	6.77± 0.22 ^{a***}
Drug	111.41± 10.22 ^{b}	53.50± 5.61 ^{b*}	29.91± 2.91 ^{b*}	70.80± 8.17 ^{b**}	10.70± 1.41 ^{b*}	3.72± 0.19 ^{b}
Zinc	105.18± 9.17 ^{b}	49.14± 5.55 ^{bc}	31.14± 3.15 ^{b*}	64.22± 7.11 ^{b**}	9.82± 1.01 ^{b*}	3.37± 0.17 ^{b}
Vanadium	10.23± 10.41 ^{b}	51.26± 5.61 ^{bc}	30.25± 3.61 ^{b*}	69.73± 7.39 ^{b**}	10.25± 1.26 ^{b*}	3.64± 0.15 ^{b}
Zinc + Vanadium	104.71± 9.99 ^{b}	48.33± 4.87 ^{bc}	32.71± 3.51 $^{a b}$	62.34± 7.42 ^{b**}	9.66± 0.99 ^{b*}	3.20± 0.11 ^{b}

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c) denote significant difference.

Table (5): The Mean values ± SD of liver glycogen, cholesterol, total lipid glutathione peroxidase (GPX) and malondialdehyde (MDA) in control and diabetic treated rat groups.

Variables / Groups	Glycogen (mg/100g)	Cholesterol (mg/100g)	Total lipid (mg/100g)	GPX (µg/g)	MDA (nmol/g)
Control (-ve)	11.70± 1.33 ^{a}	3.11± 0.33 ^{b}	30.77± 2.61 ^{c}	39.17± 2.69 ^{a}	38.41± 3.47 ^{c}
Control (+ve)	4.61± 0.48 ^{b***}	6.14± 0.96 ^{a***}	47.45± 4.67 ^{a***}	26.11± 2.71 ^{c***}	78.12± 8.22 ^{a***}
Drug	9.14± 1.41 ^{a}	4.22± 1.13 ^{b}	39.14± 3.78 ^{b*}	33.20± 3.17 $^{a b}$	50.12± 5.11 ^{b**}
Zinc	8.99± 1.11 ^{a}	4.11± 0.88 ^{b}	40.21± 4.20 ^{b*}	37.11± 3.21 $^{a b}$	43.60± 7.36 ^{b**}
Vanadium	9.01± 1.06 ^{a}	4.31± 0.77 ^{b}	38.71± 4.01 ^{bc}	35.21± 3.16 $^{a b}$	46.51± 4.61 ^{b**}
Zinc + Vanadium	9.89± 1.05 ^{a}	4.01± 0.55 ^{b}	35.71± 3.421 ^{bc}	37.33± 3.60 $^{a b}$	43.22± 4.25 ^{b**}

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c) denote significant difference.

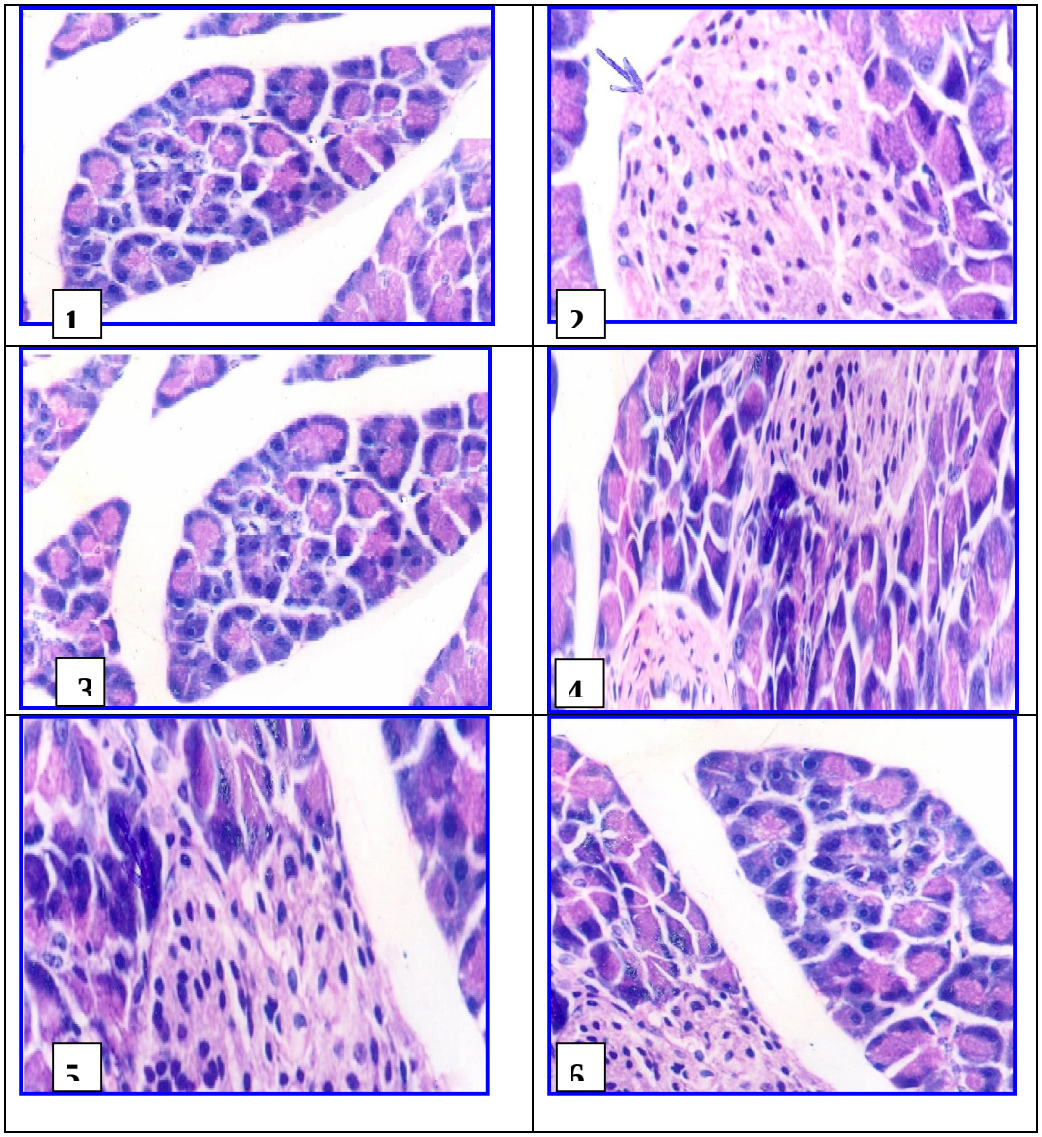

Fig (1) : Histopathological examination of the pancreas

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Economic crisis in Guilan textile industry

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Abstract: Today, industrial development is account as one of means and area for the economic development and improvement of the countries which some of the industrial courses that exist in any country due to relative advantages are account of high priority in industrial development area. texile industry, is account as the most important and oldest industry of the country and Guilan province. It can play a role as the main base of industry and mine sector if there is the required support from producers . But still it is not taken place a remarkable activities as developmental region planning in Guilan and it could not find a scientific and professional figure. So, the main goal of this article is identifying the variables and tensor factors in the Guilan texile industry and turning ways from current situation to modern developmental situation . This research had been attempted to study how turning out of the created crisis aiming to identify the crisis in texile industry and also allocating the optimal resources. The results indicated that the most important and significant problem of texile industry in Guilan is weak in management area .
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keywords : crisis, economy, texile industry, private sector, technology

1. Introduction :

In the recent decade . as south-eastern Asia countries had been progressed in economic area , Iran is continuing to its slow movement as it was before and so texile industry had been not excluded in this way.

Texile industry in Iran, despite of it's extension , power and long and great background , could not develop as the other industry elements such as machinery wear, raising production cost, raw material and inefficient management had been caused to this industry lost its competitive capacity in the world market and it is lost even 30% of domestic consumption market because of irregular imports(Alipour,2006).Texile industry is very prosperous and have a long history in Guilan and it is at 6th rank in the world . In the past , despite of its extent and bright history , This industry was one of the best industrial unit due to production and its producted fabric from quality point of view was at the extent that it exported to some countries such as America(Alipour,2007). But after the revolution , especially just in 2000-2003 few factory were active and the other factories because of enormous reason suffered from backwardness and crisis. Present problem in this industry had been rooted in its all structure widely .

Now, It is necessary the industry officials deal with the global economy with knowledge management view, especially to the texile industry of developed countries and capable and efficient management which have full knowledge on the modern technology in this industry so that they could change this wear and critical industry to a developed and efficient industry due to technology and management. Despite some problems which are in this industry , texile industry have important contribution in the value added and industrial products value of the country and due to the human forces occupying in this section , its important role in Iran economy is not negligible.

This research had been performed with an economic approach aiming to identify crisis in Guilan texile industry . For this purpose, the following special aim is considered :

1- To study the sellers market of Guilan texile industry products in the home and foreign markets .
2- To study the management role in shutting down Guilan texile factories.
3- To study the industry situation in Guilan.
4- To study industry situation in Guilan after the technology and … in forming the crisis of this industry and going out of this situation.

2. Research Method

With regard to the subject and the research essence, research method is descriptive (measurement). This research using various questions from the managers and practitioners and the available resources in statistics deal with the crisis reasons in Guilan texile industry and also it had been used from interview as a research tool for detailed study of texile crisis .

Analyzing method of data

This research had been used from descriptive and inferential statistics method for analyzing the information and in order to take results, with fish Bool technique discussed with the experts and the industry managers .

Research finding

Industrial development trend in the most developed countries showing the place and role of texile industry in the economic development process of the countries .

In Iran , texile industry is facing with human forces inflation and many of this forces are inefficient and unskilled which great part of the fixed cost allocated for their payroll .

Unfortunately, on the one hand, in this industry, the products are sold hardly and on the other hand selling prices receive in long time and there is not the possibility of raising selling rate proportion to increase the cost (Ghalamzan, 2006). Consequently the liquidity of the company suffered from reduction, repeatedly.Moreover,with regard to taking restrictive policy and restriction of bank facilities and lacking for company financial circulation, taking heavy securities, existence of excessive outstanding debt, these companies compensated their liquidity lacking with the highest cost (Safarzade,2001).

It has to be noted that irregular good's imports with high optimal quality and conform with customer taste, performing after-sale services, providing new and fashion products, on-time goods delivery to customer, high effort in marketing and market policy area and wants, had been faced the fate of Iran texile products include Guilan with a serious change and crisis.

With investigation the current situation of texile industry, we come to this important conclusion that the most major problem is machinery obsolescence. By average. This industry has a history about 35 years which itself had been caused to fading and obsolescence of the units (Karvani, 2009).

Technological problem and restriction had been caused to limitation in diversity of the products. And, on the other hand because a need for providing spare part from the abroad , it had been turned the texile industry to a dependent industry which of course, This dependent can attribute to raw materials(Fahimi,2000). Despite having raw material with ideal quality , because of expensive and lacking the machinery for transforming the material Iran has to import raw material which almost have not good quality and impose high cost on the country economy(Masoudfar,2002) .

The price of all products in texil industry is increasing and this increase is not transformable to sales price and so the interest of products will be reduced. The reasons for raising cost price briefly are : raising cost price of imported material and petrochemical material, aggregation of municipality finance duties and taking 3% (percent) of duties from factory production rather than its interest, inflation in the salary of labour and raw material, remarkable increase of fixed cost such as power, frequency and increase of receive duties type, low productivity, high waste, exchange rate (which had been caused to some problem in foreign loan refund manner)

Today, texile industry is facing with two serious and main foreign problems:

1- Intensive global competition for products with superior quality and lower price and serious attention to cost reduction component.
2- Fundamental and suddenly changes in technology area which reduced actual service life of machinery , intensively .

Constantly, exports need to along with providing inter-need in producers work order , but there are some problems and restriction on the road of producers which lead to absence of them in the global market and remove their competition power.

The most important problems and restrictions are:

Instability in the decisions and rules related to non petroleum export and in turn lacking long term planning for export development because of constant changes in exchange regulation and lacking constant policy making in this area.

Lacking concession of 100% exchange from exports to producers for importing raw material and the other requirement of industry section.

Unjusting and uneconomic of exchange rate for exports with compared to exchange cost price for production .

Weakness of Iran export development center in holding an exhibition which as a result of presence in this exhibitions for Iranian producers and exporters , it lead to just a set of overhead and excessive cost(Nasrollahi,2000).

The main problem in the texile industry is lacking investment in the important section of this industry with regard to the existence problem and inefficient of many units, investment in the main section of the industry have not economic justification and even have operating loss. Also the current condition in the investment on Guilan texile

industry is not suitable. Moreover, there was not an exact and scientific view on management problem in Iran community from long before. Experts had been stated that the most important element for creating this problem are inability of managers in taking strategic decision, lacking performing authority and decision- making, multicareer and unfamiliar with their roles. According to all economic exports in Guilan, The most important and great problem in texile is weakness in management section. The loss from the heavy snow crisis in 2004 in Guilan which caused to high damage to 400 big and small unit which was from 30 to 100% is the other problem of texile industry in this province.

Table No(1) is investigating the number of Guilan texile industry factory relative to total number of industrial factory from 2001 to 2006 . In this viewing, the number of Guilan texile industry factories had been descending order since 2007 respectively and showing weakness of texile industry section relative to other economic section.

Table 1: The number of Guilan texile industry factories and comparing them with total industry factories of the country during 2007-2006.

Year	The number of texile industry factories	The number of industry	Texile contribution in the industry
2001	2907	13509	21.5
2002	2665	16257	16.3
2003	64	610	10.4
2004	63	564	11.1
2005	56	565	9.9
2006	52	565	9.2

Source : Guilan statistical year book, various year

4. results and Discussion

Most texile industry plant from 22 years ago up to now which were under the government support, now are living in crisis situation. Their various debts are so high which are included in article 141 due to trade law. Up to now, the performed efforts had been cross section. For this units can locate in the correct path with a scientific and rational planning , the only possible way is performing article 44 of constitution (privatization).

At the present which the community is in dangerous situation because of unemployment of young generation , we can create constructive productive movement through attracting their participation in managing the crisis units .

With regard to all mentioned problems about Guilan texile industry, the role of medium in moving crisis due to effectiveness of TV must be attended. In this regard, providers put texile industry in the high priority, because this industry always had a intensive dependence and belonging from Guilanian people and have bright and long history in Guilan. Preparing some programs such as round table, representation, report, documentation and … which take advantages from hard research support are helpful in the developmental trend of this industry and helping to remove its crisis.

Current research could have key role in constructing TV programs in order to inform people about texile industry .

High structural changes toward economical section indicate suitable production situation and there are more powerful basis for development and improvement in it. In all industrial developed countries, governments take some policies in favor of producers in order to support their economy, trade and their exports and meanwhile, they overlook from their right in order to support the producers. In Iran, this story is different and in fact this is producers who support government and the condition is a form that it tends to imports side itself. In fact lacking government subsidy is itself encouraging the imports. The country economic trustees in imports raw material which is the key factor in the production are performing much severity , while in entering final product which include domestic market, There are not exercising tax and customs policy(Rodas,1998) .

In fact , in our country , as much as our export is an important subject, import is take place easily. Performing liberation policy to found industrial units had been faced with extremes , and country capital and exchange (which along with foreign debt, associated with heavy pressure on the country economy) had been invested without scheduling . So in some part of the industry (main group of texile products) we are encountering with out of order capacities because of need to more investment and at the other section (subgroup of products) which had been found new productivity capacities, we are facing with deficiency productivity from capacities.

Subgroups need to few initial investment volume for establishing new units . So the investors had been paid more attention to this units and this lead to raise inflation in this section which sometimes had surplus production capacity and at the present it remained useless and at least 1/3 of the performed investment are in this section . It

should be noticed that allocating and dividing the capital and exchange equilibrately between the various parts of texile industry had been not taken place . Also, because of lacking export power there are some difficulties and current problems in this part and if it occurs, remarkable exchange could reach to the country(shokouhi,2000) .

Economic experts believe that increasing and development of exports will lead to raise investment, Improving production level, and promoting technology of texile industry . So, some actions such as granting export rewards, can improve the quality of manufactured commodities and return the lost market of texile industry (Balassa, 1965) .

The important challenges faced to this industry is demand which must be solved with developing export market, creating regional cooperation and removing discrimination tariff between domestic and foreign producers .

There is a need to exact and solid scheduling for development and modernization of texile industry due to economic and making employment. The necessary attempts in this regard are as follow:

1- using from Islamic development bank facilities
2- selecting and assignment competence and expert managers
3- creating closeness possibility or participation of texile factories with developed countries industry .
4- creating research and laboratorial environment in the texile factories for updating factory products due to quality progressing .
5- Improvement and revision in banks management and pay credit manner and providing facilities to the texile industry .
6- Training expert engineers in universities
7- Lowering social security insurance rate for texile factories and lateral industry from 30% to 18% .
8- Revision in high rate of bank interest
9- Interaction of employment department and industry and mine department with universities and factories for providing apprenticeship period of students.
10- Adjustment customs duties for importing goods which are not produced in home and it is necessary for industry.
11- Giving exchange term loan with low interest for purchasing the equipment and providing liquidity.
12- Providing necessary possibilities for texile course students for coordinate the theory learned lessons with practice.
13- Tax exemption of texile factories for consecutive 10 yeas .

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Effects of Aldosterone Receptor Antagonist on Vascular Calcification and Bone Disorder in Streptozotocin-Induced Diabetic Rat

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Abstract: Background: Vascular calcification and bone disorders are increasingly recognized problems in patients with diabetes. Calcium dyshomeostasis is a major risk factor for cardiovascular morbidity and mortality. Diabetic osteoporosis seems to be dependent on qualitative and quantitative alterations of the bone, as well as microangiopathic complications of diabetes mellitus. Aim: We investigated calcium dyshomeostasis, and bone histological and metabolic abnormalities in Streptozotocin-induced Type 1 Diabetes Mellitus in rats. The possible role of the aldosterone receptor antagonist, spironolactone, in reversing these effects was assessed. Materials and Methods: Adult Female Wistar rats were divided into three groups: Control group, Streptozotocin-induced diabetic group (STZ-D), and Aldosterone-receptor antagonist-supplemented diabetic group (ARA-STZ). Diabetes was induced by a single intraperitoneal injection of streptozotocin, 40 mg/Kg BW. Spironolactone (aldosterone receptor antagonist) was given by oral gavage in a daily dose of 15 mg/kg BW for 4 weeks. At the end of the experiment, serum levels of calcium, phosphate and alkaline phosphatase were evaluated. Histological examination of the tibia was performed, together with analysis of renal vascular calcification and Immunohistochemistry for inducible nitric oxide synthase (iNOS) in renal tissue specimens. Results: STZ-D rats showed normophosphatemia and significant hypercalcemia with significantly increased serum alkaline phosphatase compared to control group. Bone loss was also observed. Histological examination of the small renal blood vessels showed calcification in the walls, as well as, reduction in iNOS immunostaining. These metabolic and histological abnormalities in STZ-D rats were remarkably corrected by the administration of spironolactone. Conclusion: The current results underscore the important role of aldosterone in promoting vascular calcification and osteoporosis in diabetic rats and the potential role of aldosterone receptor antagonist, spironolactone, in correcting these clinical problems in diabetic rats.
[Shadia A.E. Barakat, Nermine K.M. Saleh, Sahar S. Thabet, Hanan A. Saleh and Abd El-Hamid A. Mohamed. **Effects of Aldosterone Receptor Antagonist on Vascular Calcification and Bone Disorder in Streptozotocin-Induced Diabetic Rat.** Journal of American Science 2011;7(1):422-430]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: Ca^{2+} homeostasis, osteoporosis, vascular calcification, DM, Aldosterone.

1. Introduction:

Diabetes mellitus (DM) is associated with increased cardiovascular mortality compared to the general population. The etiology of the increased mortality is multi-factorial, but recent data supports that vascular calcification may be a contributing factor (Al-Aly, 2007). In agreement, Chen *et al*. (2006) reported that vascular calcification is more common in patients with DM compared with the general population and is associated with increased mortality, stroke and amputations.

Studies on skeletal involvement in patients with DM have generated conflicting results, largely because of the pathogenetic complexity of the condition (Carnevale *et al*., 2004). Cohort studies carried out on large samples indicate that diabetic patients (with both type 1 and type 2 disease) have a higher risk for fracture, in particular for hip fracture, the most dangerous osteoporotic complication. This seems to be dependent both on qualitative and quantitative alterations of the bone, as well as on extra-skeletal factors due to, for example, microangiopathic complications of the disease (Carnevale *et al*., 2004).

Also, studies on bone mineral characteristics in children with type 1 DM have generated conflicting results. Many studies have shown that children with type 1 DM are at risk of having a decrease in bone mass (Valerio *et al*., 2002, Heap *et al*., 2004 and Moyer-Mileur *et al*., 2004), whereas other studies found no bone mineralization abnormalities in diabetic children (Pascual *et al*., 1998, De Schepper *et al*., 1998 and Liu *et al*., 2003). However, diabetic osteoporosis is increasingly recognized as a significant co-morbidity of type 1 DM (Thrailkill *et al*., 2005).

Giachelli *et al*., (2005) reported that local environmental cues such as disturbances in phosphate or calcium levels control vascular calcification that is associated with vascular smooth muscle cells (VSMCs) phenotyping modulation. VSMCs are implicated to have the capacity to undergo

modulation from a contractile to an osteochondrogenic phenotypic state.

The classic understanding of aldosterone as a hormone produced by the adrenal cortex and involved in the reabsorption of sodium and the secretion of potassium and protons in the collecting duct needs to be extended. Aldosterone is found to be generated in many other tissues besides the adrenal cortex; the most convincing evidence relates to the central nervous system. However, suggestions that aldosterone is produced in the heart remain controversial (Connell and Davies 2005). Recent studies have begun to explore the molecular mechanisms of the direct actions of aldosterone on the cardiovascular system. They have demonstrated the expression of functional mineralocorticoid receptors (MRs) in the heart, large arteries, VSMCs, and endothelial cells (Nishiyama *et al*., 2009). Recent human and animal studies suggest that activation of the MRs by aldosterone causes diabetic complications such as; microvascular damage, vascular inflammation and endothelial dysfunction (McFarlane and Sowers 2003). Aldosterone is capable of inducing cardiac fibrosis characterized by enhanced accumulation of collagen and increased fibroblast proliferation *in vivo*. In a previous study, we demonstrated that a low-dose MRs antagonist therapy (Spironolactone) markedly reduced cardiac fibrosis in diabetic rats (Saleh and Saleh, 2005). Moreover, recently, Aldosterone has been reported to play a relevant role in vascular calcification (De Solís *et al*., 2008). MRs in VSMCs, in response to aldosterone, modulates expression of osteogenic genes including alkaline phosphatase (ALP) stimulating vascular calcification (Jaffe *et al*., 2007).

The reported benefits of aldosterone receptor antagonists (ARA) in diabetic nephropathy, despite reported normal or low levels of plasma aldosterone, suggest that this hormone may be produced locally within the kidney (Xue and Siragy, 2005) and this local renal production of aldosterone was confirmed by Taira *et al*. (2008). The local renal aldosterone system is regulated by insulin-deficient diabetic hyperglycemia (Xue and Siragy, 2005). In animals treated with streptozotocin (STZ), a significant increase in renal aldosterone synthase mRNA was reported (Xue and Siragy, 2005).

From the above mentioned data we hypothesize that elevation of aldosterone in type 1 DM could play a role in Ca^{2+} dyshomeostasis and vascular calcification. Thus, interruption of the system would be expected to produce parallel improvement in vascular and bone outcome. Many human and animal studies suggest that angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) are unable to provide long-term aldosterone suppression (Naruse *et al*., 2002 and Otani *et al*., 2008). Therefore, we hypothesize that aldosterone antagonism could be a rational therapy rather than ACE inhibitors.

So, this work was designed to clarify the possible role of aldosterone antagonist on Ca^{2+} dyshomeostasis and vascular calcification in type 1 DM.

2. Material and Methods:

This work was performed on 17 female Wistar rats aged 12-14 months. The rats were maintained under standard conditions of boarding. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. Rats were allocated into 3 groups: a) Control group, injected intraperitoneally (i.p) with citrate buffer (n=6). b) Streptozotocin-induced diabetic (STZ-D) group, (n=5). c) Aldosterone-receptor antagonist-supplemented diabetic group (ARA-STZ) (n=6); where diabetic rats were orally supplemented with spironolactone, an aldosterone receptor antagonist (15 mg/Kg BW), by gavage for 4 weeks starting 3 days from induction of diabetes, when these rats were proved to be diabetic.

Induction of diabetes:

Rats destined to be diabetic were given a single intraperitoneal injection of STZ (Sigma) in a dose of 40 mg/kg body weight, dissolved in 2 ml of 0.05 M citrate buffer according to Yoshino *et al*, (1992). Because of unstable nature of STZ, it was freshly dissolved 10 minutes just prior to injection. Citrate buffer was prepared by adding 47 ml of 0.05 M citric acid (Vabberlin - chemic) to 53 ml of 0.05 M trisodium citrate dehydrate (Adwic). Both were prepared according to Dawson *et al*. (1986). pH of citrate buffer was adjusted exactly at 4.5. Rats received 5% sucrose solution for the first 48 hours after STZ injection to minimize deaths from hypoglycemia. After 72 hours from STZ injection, tail blood samples were obtained by sharp cut and blood glucose concentration was determined by using glucose strips supplied from Boehringer Mannheim using Reflolux S-type 117 2115 6 V apparatus in order to ensure successful induction of diabetes in STZ-treated animals. Rats having blood glucose below 200 mg% were considered non-diabetic and were excluded from further study. STZ-induced diabetic rats had free access to food and water. Blood glucose was reestimated before sacrifice and only rats with blood glucose above 200 mg % were included in the study.

Experimental procedures:

On the day of sacrifice, overnight fasted rats were weighed and anaesthetized with thiopental sodium 40 mg/kg intraperitoneally (Sandoz GmbH, Kundl-Austria). Blood sugar was tested using glucose strips. The blood samples were collected from the abdominal aorta then centrifuged, and the serum was stored at -20°C for biochemical analysis.

Biochemical determinations:

Serum calcium, phosphate and alkaline phosphatase levels were measured using standard laboratory methods.

Histological examination of bone:

Bone segments (proximal end of tibia) were removed. The tibias were dissected out, fixed in 10% buffered neutral paraformalehyde, and decalcified in EDTA solution for 2 weeks. Once decalcified, the specimens followed routine histological processing and paraffin embedding was done. Paraffin sections (5µm thick) from the metaphysis of the tibias were deparaffinized and stained by haematoxylin & eosin (H&E) (Bancroft and Gamble, 2002) for light microscopic examination.

Histochemical detection of calcium in small renal blood vessels:

Kidney specimens were fixed in 10% buffered neutral paraformaldehyde solution, embedded in paraffin, and deparaffinized by standard procedures. Thin sections (5 µm) were stained by Alizarin red S stain (Bancroft and Cook, 1994) for demonstration of calcium deposits.

Immunohistochemistry for iNOS:

Kidney specimens were stained with polyclonal anti-inducible NOS (iNOS) antibodies. Immunohistochemical localization of iNOS protein was performed based on the method previously described by Kawaguchi *et al*. (2001).

3. Results:

Table 1: Serum levels of calcium, phosphate and alkaline phosphatase (ALP) in: Control, Streptozotocin-induced diabetic rats (STZ-D), and Aldosterone receptor antagonist-supplemented diabetic rats (ARA-STZ rats).

Parameters	Control	STZ-D	ARA-STZ
Calcium (mg/dl)	6.4±0.39 (6)	9.8±0.89[a] (5)	5.5±0.66[b] (6)
Phosphate (mg/dl)	5.7±0.40 (6)	5.5±0.25 (5)	6.3±0.52 (6)
ALP (IU/liter)	188.6±13.3 (6)	402±69.8[a] (5)	779.2±77.5[a,b] (6)

Data are presented as means ± SEM.
In parenthesis is the number of observations
(a) Significance calculated by least significant difference (LSD) at < 0.05 from control group.
(b) Significance calculated by LSD at < 0.05 from STZ-D group.

Serum levels of calcium, phosphate and alkaline phosphatase (table 1):

The results of this study clearly demonstrated that the serum Ca^{2+} level was significantly elevated 4 weeks after the induction of diabetes in STZ-D rats as compared to the control rats. This increase was significantly inhibited by administration of spironolactone to the ARA-STZ rats. Serum inorganic phosphate level was insignificantly changed among all studied groups. Serum alkaline phosphatase was significantly increased in the STZ-D rats as compared to the control rats. Spironolactone administration resulted in a more significant rise of serum alkaline phosphatase in the ARA-STZ rats as compared to both the control and the STZ-D rats.

Histological examination of the bone:

Examination of sections from the control group revealed that the proximal metaphysis of the tibia was formed of an outer shell of compact bone (cortical bone) and inner trabeculae of cancellous bone. The compact bone consisted of outer, inner and interstitial bone lamellae as well as Haversian systems. Inbetween the lamellae osteocytes resided in their lacunae. The shell of compact bone was covered by periosteum and lined by endosteum.

Cancellous bone was formed of a network of bone trabeculae composed of irregular bone lamellae between which osteocytes resided in their lacunae. The endosteal surface of trabeculae was lined by osteoprogenitor cells, osteoblasts and osteoclasts. Bone marrow spaces were seen between the trabeculae (Fig. 1).

Bone sections from STZ-D rats revealed thinning of the outer cortical bone as well as the inner cancellous bone trabeculae as compared to the control group. The cancellous bone trabeculae lost their normal architecture and appeared as discontinuous bony ossicles separated by widened bone marrow spaces (Fig. 2).

Bone sections in ARA-STZ rats revealed marked improvement as compared to those of the STZ-D rats. Cortical bone thickness was very similar to the control group. The cancellous bone trabeculae partially regained near normal structure and appeared more continuous, with less widened bone marrow spaces (Fig. 3).

Histological detection of calcium in small renal blood vessels:

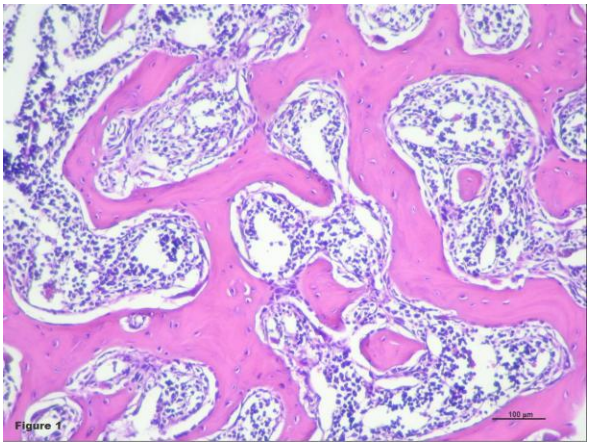


Fig. (1): Photomicrograph of proximal metaphysis of tibia of the control group showing well-formed network of trabeculae of cancellous bone. (H&E X250)

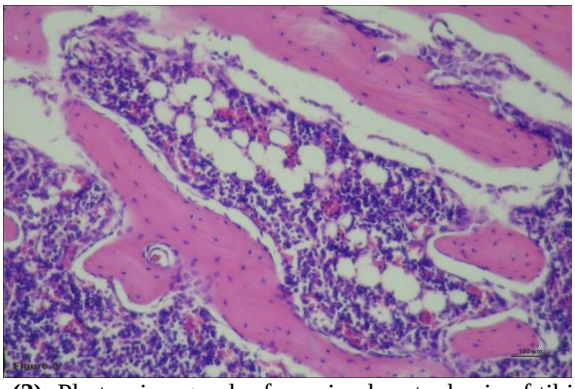


Fig. (3): Photomicrograph of proximal metaphysis of tibia in ARA-STZ group showing preserved architecture of trabeculae in comparison to STZ-D group. Trabeculae appear more or less continuous with bone marrow spaces inbetween. (H&E X250)

Examination of the small blood vessels of kidneys of the control rats revealed no calcium deposits (Fig. 4). The kidneys of STZ-D rats revealed calcium deposits in the small blood vessels, detected as red lines in both cortex and medulla (Fig. 5). Group ARA-STZ revealed minimal calcium deposits in the small blood vessels of the kidneys (Fig. 6).

Immunostaining of the small renal blood vessels for iNOS:

In the control group, iNOS immunostaining was densely detected in the endothelium of the peritubular blood capillaries (Fig. 7). In the STZ-D rats, iNOS immunostaining was markedly reduced in the endothelium of the small blood vessels as compared to the control group (Fig. 8). In the ARA-STZ rats the iNOS immunostaining was increased as compared to the STZ-D group (Fig. 9).

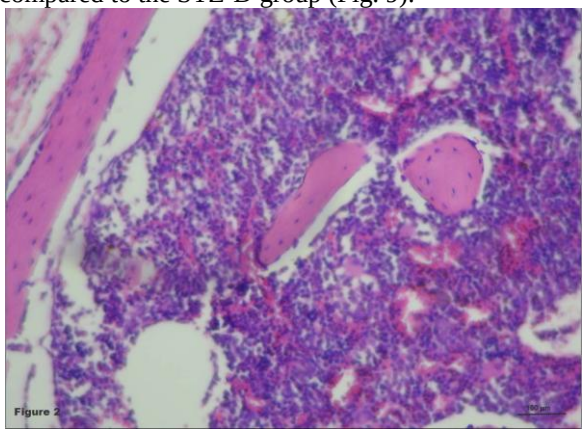


Fig. (2): Photomicrograph of proximal metaphysis of tibia of STZ-D group showing loss of the normal architecture of trabeculae of cancellous bone with widening of bone marrow spaces. (H&E X250)

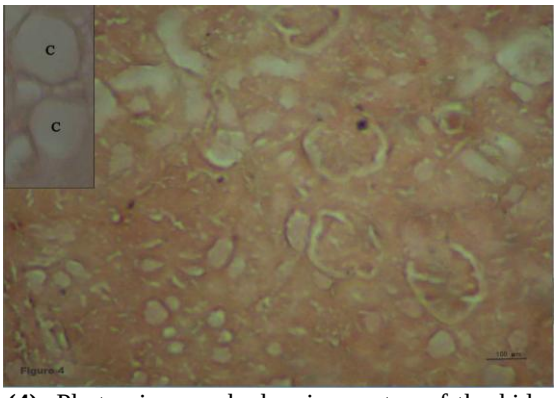
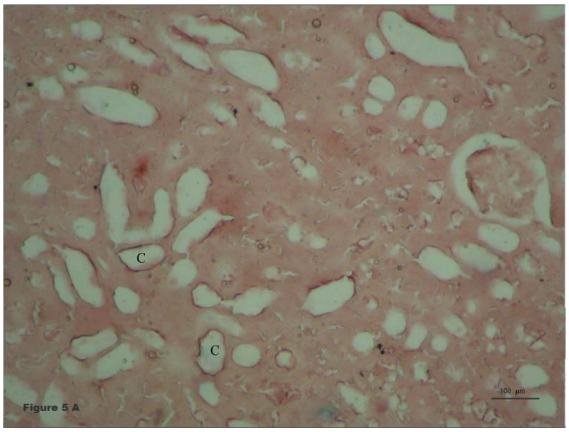
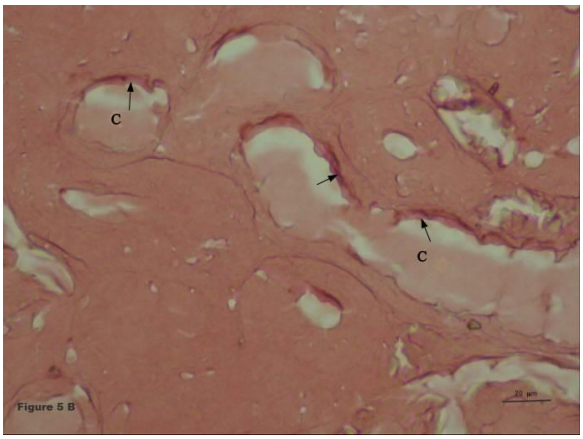


Fig. (4): Photomicrograph showing cortex of the kidney of the control group. No calcium deposits are detected in the peritubular capillaries. (Alizarin red S stain X250)
Inset: Higher magnification showing two capillaries (C) with no calcium depostis (Alizarin red S stain X640)



A



B

Fig. (5): Photomicrograph showing cortex of the kidney of the STZ-D rats.
A: Calcium deposits can be detected in the wall of the small blood vessels (C) (Alizarin red S stain X250)
B: higher magnification showing calcium deposition (↑) in the wall of the renal blood vessels (C)(Alizarin red S stain X640)

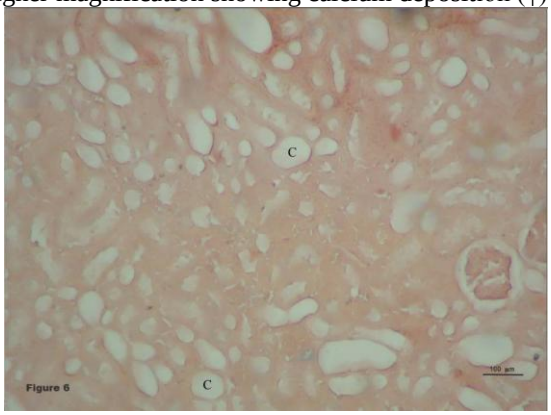


Fig. (6): Photomicrograph showing negligible calcium deposits in the small renal blood vessels (C) in ARA-STZ rats. (Alizarin red S stain X250).

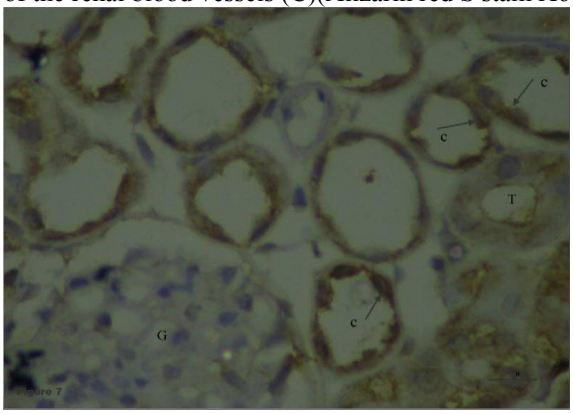


Figure (7): Photomicrograph of iNOS immunostaining of peritubular capillaries from control rats showing dark brown densely stained areas (↑) in endothelial cells (G: glomerulus; T: tubule; C: capillary). (iNOS X 640)

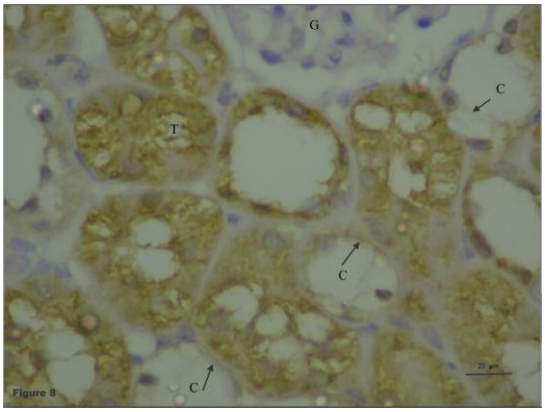


Fig. (8): Photomicrograph showing faintly stained endothelial cells (↑) in peritubular capillaries for iNOS in STZ-D rats (G: glomerulus; T: tubule; C: capillary). (iNOS X640)

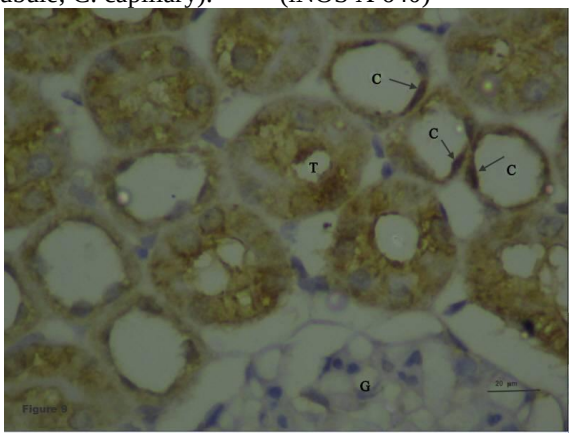


Fig. (9): Photomicrograph showing moderate iNOS-staining of endothelial cells (↑) in peritubular capillaries of ARA-STZ rats (G: glomerulus; T: tubule; C: capillary). (iNOS X640)

4. Discussion:

The current study clearly demonstrated dysregulation of Ca^{2+} metabolism in STZ-D rats. Serum Ca^{2+} level was significantly elevated 4 weeks after induction of diabetes, histological examination of bone revealed osteoporotic changes, and histological examination of renal blood vessels revealed calcification of small renal blood vessels, together with reduced iNOS staining. Treatment with spironolactone, an aldosterone-receptor antagonist, reduced all the previously mentioned findings.

The increased serum Ca^{2+} concentration in STZ-D rats could result from the release of Ca^{2+} from bone tissues. Several mechanisms could underlie osteoporosis observed in STZ-D rats. Advanced glycation end products (AGEs) had been reported to contribute to poor bone strength, and increased receptors for AGEs (RAGEs) were manifested in chemically-induced diabetes in mice (Thrailkill *et al*., 2005). RAGE, a multiligand receptor, contributed to the pathogenesis of diabetic complications through regulating osteoclast maturation, function, and bone remodeling (Zhou, *et al*., 2006). Osteoclasts are multinucleated, terminally differentiated cells from hematopoietic monocyte/macrophage precursors, whose bone resorption activity is critical in regulating bone mass (Zhou, *et al*., 2006). The increase in oxidative stress (Johansen *et al*., 2005) together with the increase in proinflammatory cytokines reported in diabetes (Barakat *et al*., 2008) have been recognized as contributing factors to enhanced osteoclast activity (Sheweita and Khoshhal, 2007).

Insulinopenia in STZ-D rats could be a contributing factor to the osteoporosis observed in these rats. Several lines of evidence from in vitro bone cell cultures support the idea that insulin can exert direct anabolic effects on bone cells. The lack of insulin in patients with type 1 DM, may be disadvantageous for osteoblast number and activity and collagen formation (Botolin *et al*., 2005). In addition to the direct effects of insulin on bone cells, insulin was reported to exert synergistic effects with other anabolic agents in bone, such as insulin-like growth factor-1 (IGF-I) (Thrailkill *et al*., 2005).

Elevations in plasma aldosterone level were reported in STZ-diabetic animals (Xue and Siragy, 2005). Aldosterone was reported to be accompanied by a proinflammatory state, characterized by oxidative and nitrosative stress, immune cell activation, and bone loss (Vidal *et al*., 2006 and Chhokar *et al*., 2005). Thereby, hyperaldosteronism could be another contributing factor to osteoporosis observed in STZ-D rats.

The results of the current study demonstrated correction of the hypercalcemia and osteoporosis in ARA-STZ rats. Aldosterone antagonism, by attenuating oxidative stress (Nishiyama and Abe, 2006) and reducing inflammatory changes could provide a mechanism for attenuation of osteoporosis observed in the STZ-D rats.

The observed elevation of ALP in ARA-STZ rats could reflect increased osteoblast activity as histological examination of bone revealed apparent improvement in thickness and continuity of trabeculae.

At the same time, histological examination of blood vessels revealed calcification of small renal blood vessels in diabetic rats. Multiple factors could be suggested to play a role in the pathophysiology of vascular calcification in STZ-D rats. Chen *et al*., (2006) reported that high glucose level could induce or modulate phenotypic transformation of VSMCs to osteoblast-like cells with subsequent mineralization in vitro.

In a previous study, we reported significant increase in tumor necrosis factor-alpha (TNF-alpha) in the STZ-D rats (Barakat *et al*., 2008). TNF-alpha was reported to increase calcium deposition, and induce the expression of osteogenic signals like ALP (Al-Aly *et al*., 2007). In agreement, we reported in the current study a significant increase in serum ALP in the STZ-D rats. At the same time, it was reported that abnormalities in mineral metabolism that enhance the calcium x phosphate product, as the hypercalcemia encountered in STZ-D rats in our results, could exacerbate vascular calcification (Giachelli *et al*., 2005).

Aldosterone was reported to regulate the expression of several genes that have been implicated in vascular calcification including the ALP gene (Jaffe *et al*., 2007). Aldosterone was found to promote mineralization of calcifying VSMCs in a MR-dependent manner. These observations suggest that one of the clinical effects of MR antagonists (as spironolactone) may be inhibition of this MR-mediated vascular calcification (Jaffe *et al*., 2007). This could explain the present findings of increased vascular calcification in the untreated diabetic group and its reduction with spironolactone treatment.

Moreover, Aldosterone induces the generation of reactive oxygen species (Rüster and Wolf, 2006). Studies showed that hydrogen peroxide enhanced bone markers expression in VSMCs implicated in osteoblastic differentiation (Sutra *et al*., 2008).

Jaffe *et al*., (2007) reported that endothelin-1 (ET-1) synthesis is elevated during aldosteronism. ET-1 induced oxidative and nitrosative stress and expression of adhesion molecules in the affected vasculature in rats with chronic stress of mineralocorticoid.

Nitric oxide (NO) is a messenger molecule produced by the NO synthase (NOS) isoforms. Inducible NOS (iNOS) isoform can be found in the vasculature, in VSMCs. iNOS was found to inhibit VSMCs calcification. These data suggest that NO regulates vascular calcification (Kanno *et al*., 2008). Substantial evidence indicates that diabetic vascular dysfunction is associated with marked alterations of NO pathways. In the current study, iNOS-like immunostaining was decreased in the endothelium of small renal blood vessels in the STZ-D rats. This result is consistent with a previous finding in a study which reported that high glucose reduces cytokine-induced iNOS activity in VSMCs (Muniyappa *et al*., 1998). This decrease in vascular iNOS immunostaining was reflected on the increased vascular calcification in the untreated diabetic group.

Endogenous iNOS expression and activity have key function in increasing endothelial survival and maintaining function. Thus, suppression of iNOS was found to significantly contribute to endothelial dysfunctions during oxidative stress (Hemmrich *et al*., 2003).

Therefore, in the present study, increased inflammatory cytokines, diminished iNOS activity, elevated serum aldosterone, high blood glucose and high calcium levels could provide stimulatory signals for the observed vasculopathy in the STZ-D rats.

The vasculopathy observed in the STZ-D rats was clearly ameliorated in the ARA-STZ rats. In the current study, iNOS-like immunostaining was enhanced in the ARA-STZ rats. Induction of iNOS could have reduced pathological vascular calcification.

In the vascular system, MR could be localized in endothelial and VSMCs. Aldosterone induces oxidative stress in vascular cells through NADPH oxidase activation. MR antagonist decreases oxidative stress and increased NO bioavailability (Bauersachs and Fraccarollo, 2006).

Taken together, in the current study aldosterone antagonism could prohibit vasculopathy in ARA-STZ rats by its antioxidant effects, increasing NO bioavailabilty, decreasing expression of vascular osteogenic signals, as well as normalization of blood calcium levels.

From the aforementioned data, our findings draw attention to a significant role of the aldosterone hormone in the vascular calcification & osteoporosis; two significant complications that accompany DM type 1. In this study, we presented the evidence supporting the potential benefits of early blocking the actions of aldosterone in diabetes in lessening the burden of vascular and bone effects in this metabolic disorder.

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The effects of peer education on health behaviors in girls with dysmenorrhea

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Abstract: This study was conducted to compare the effect of peer-led VS health-provider-led self-care education on dysmenorrheic girls' knowledge, attitude, and menstrual symptoms of primary dysmenorrhea at dormitories of Ferdowsi University in Mashhad, Iran. In this randomized clinical trial, 165 girls between ages 19-25 who had experienced menstrual cramps three or more times during the last six months were randomly assigned to three groups (peer-led self-care education, health-provider-led self-care education, and control). A Menstrual Knowledge Questionnaire (MKQ), Menstrual Attitude Questionnaire (MAQ), and Menstrual Information Form were the main instruments in this study. Data were collected in the baseline menstrual period and one and two menstrual periods after intervention. One-way ANOVA and Kruskal-Wallis were used to analyze data by SPSS software. Menstrual Knowledge in the peer-led self-care education group increased 2.1 times and 2.5 times in the health-provider-led self-care education groups. Negative concepts of menstrual attitude decreased in the peer-led self-care education group (56.6 vs. 40.2, p=0.009) more than the health-provider-led self-care education group (56.9 vs. 48.3, p=0.035). There was no significant difference in the measure of decrease in pain score between interventional groups at both the first (p=0.988) and second (p=0.965) menstrual periods after intervention. These findings provide preliminary evidence that peer education can be effective health promotion in primary dysmenorrheic girls.
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1. Introduction

The prevalence of primary dysmenorrhea, which is the most common gynecological problem among menstruating young women and adolescents, has been reported in many studies to vary between 50 and 90 percent (Latthe, 2006). Moreover, it was reported more than 70 percent in Iran (Panahande, 2008; Afshari, 2006; Jalili, 2004; Kamjo, 2001).

Typically, when women visit health providers for dysmenorrhea, they prescribe non-steroidal anti-inflammatory analgesic drugs or oral contraception, but neglect to emphasize the importance of dysmenorrheic self-care. From the viewpoint of health promotion, women's self-care should be enhanced and medicalization should be reduced (Kumar, 2004). Self-care behaviors related to dysmenorrhea refer to those actions taken to enhance comfort and to prevent or respond to the condition (Ching-Hsing, 2004). Self-care behaviors are divided into two categories: externally oriented behavior including searching for knowledge, expression of emotions, seeking assistance, control over external factors, and internally oriented behavior including resource utilization and self-control (Orem, 1995). Self-care education is in the early stages in developing countries and hasn't made considerable progress (Rafie far, 2005). Few educational programs have been directed at improving self-care behaviors among dysmenorrheic girls. Because of the high prevalence of primary dysmenorrhea and low self-care knowledge, there is a strong need for investigation for effective strategies. Self-care agency and self-care requirement are two main concepts of the self-care theory. Self-care agency is the human capability to give self-care, and self-care requirement is an essential or desired input for an individual or the individual's environment to maintain or optimize human functioning. There is a bilateral relationship between three concepts of this theory that means reinforcement of one is due to reinforcement of others (Orem, 1995). Based on Bandura's Social-Cognitive theory, environment is the most important and indisputable factor in improvement of self-care agency. Environment provides behavioral models that can reinforce the learning process (Bandura, 1986). Peer educators act as positive role models. Peer education has grown in popularity and practice in recent years in the field of health promotion

(Turner, 1999). Research suggests that people are more likely to hear and personalize messages and thus to change their attitudes and behaviors if they see the messenger is like them and faces the same concerns and pressures (Topping, 2001). Peer groups give group members a safe place to test new behaviors and skills, promote self-awareness, self-understanding, and change as confidence is enhanced and roles are strengthened through sharing (Rejeh, 2009; Edwards, 2001). Peer education can be used with many populations and age groups for various goals (UNFPA, 2006). Education of sexual behaviors (Wyandt, 2003), reproductive health (Ozcebe, 2003), methods of AIDS prevention (Mahat, 2008), and dietary behaviors (Perez-Escamilla, 2008) are the topics addressed by peer education and combined with controversial results.

In Lorig et al.'s (2009) study, a peer-led diabetes self-care program versus the usual care group offered significant improvements in depression, symptoms of hypoglycemia, communication with physicians, healthy eating, and self-efficacy. So people with diabetes can benefit from a community-based, peer-led diabetes program (Lorig, 2009). On the other hand, Crotty et al. (2009) evaluated self-management and peer support among people with arthritis. The results of this study revealed that a peer-led arthritis self-management program didn't have significant effect on pain, function, or quality of life in the short term and further research was needed to assess peers' impact in maintaining health behaviours in this patient group (Crotty, 2009). To our knowledge, there exists no evidence for the use of peer-led self-care education in dysmenorrheic girls. Considering the importance of dysmenorrheic self-care, benefits of peer education, and the resulting relevant controversies about the effect of peer-led education in other health topics, this study was conducted to determine the effects of peer education on health behaviors in girls with dysmenorrhea.

2. Material and Methods

This study was a randomized controlled trial on 165 dysmenorrheic girls who lived in dormitories of Ferdowsi University of Mashhad, Iran, between April and October 2009.

2.1. Subject recruitment

Single girls younger than 25 years who had experienced primary dysmenorrhea based on the Verbal Multidimensional Scoring System for at least three months in the past half year and didn't have any prior history of gynecological disease and symptoms of secondary dysmenorrhea were eligible. The menstrual history for diagnosis of primary dysmenorrhea included the regularity of menstrual cycles (menstrual cycle length from 28 to 35 days and bleeding cycle length from three to seven days) and the beginning of pain, a few hours before the onset of a menstrual period, not lasting more than 72 hours. Exclusion criteria included current or recent use of hormonal contraception, refusing to participate in educational section or fill out the follow-up questionnaire.

Two-hundred-and-nine dysmenorrheic girls from three different dormitories of Ferdowsi University fulfilled the inclusion criteria and were randomly divided into three groups of peer-led self-care education (n=79), health-provider-led self-care education (n=65), and control (n=65) group. Data were collected in three stages. In the first stage, the girls were asked to provide demographic information and fill out Menstrual Information Form (MIF) during their first future menstrual cycle. After that, girls in the peer-led self-care education group participated in a meeting session. During this visit, the investigator explained the peer-education procedure and its objectives. In addition, the girls were made aware of the needed characteristics and the roles of peer-educators in the group. Then participants were asked to form small groups (six-seven persons) as peer groups and select their peer-educators themselves. Fourteen dysmenorrheic girls adopted the peer educator's role participated in "Training of Peer Educators Workshop." The objectives of this workshop were: 1- To know about the concept of peer education, 2- To introduce some basic principles and qualities required for becoming a peer educator, 3- To know about Orem's self-care theory, 4- To know about dysmenorrheic self-care behaviors including: searching for knowledge, expression of emotion, seeking assistance, control over external factors, self-control, and resource utilization. At the end of workshop, 10 participants who earned the highest score of "Peer Educator's Skills Rating Form" were selected as peer educators. In the next stage, subjects completed pre-test forms, including the Menstrual Knowledge Questionnaire (MKQ) and the Menstrual Attitude Questionnaire (MAQ). Then dysmenorrheic self-care education sessions were carried out by a midwife in the health-provider-led self-care education group and by peer educators in the peer-led self-care education group with the method of small-group discussion. In the last stage, MIF was completed for two follow-up periods and post-test forms the same as the pre-test forms were completed at the end of the second follow-up period.

2.2. Questionnaires

2.2.1 Menstrual Information Form

This form included the four components: a) Visual Analog Scale (VAS) to measure the severity of pain during the first three days of the menstrual cycle, ranging from no pain at all (0) to intolerable pain (10). The measurement of menstrual pain by VAS is common practice in research on dysmenorrhea and has been found to be reliable for estimating pain severity. b) Higham chart for assessment of menstrual blood loss is known as a valid and reliable tool for this purpose c) Daily recording schedule to determine the pattern of painkillers use during the menstrual period. d) Recording schedule of symptoms of premenstrual syndrome included 11 diagnostic criteria as described in DSM-IV-TR. In this study, girls who have had at least five of the following symptoms for most of the time during the premenstrual week are identified as suffering from PMS. Eleven diagnostic criteria were:

- Depressed mood, hopelessness
- Anxiety
- Affective ability
- Anger, irritability
- Decreased interest in usual activities
- Difficulty concentrating
- Decreased energy
- Appetite changes or cravings
- Changes in sleep
- Feeling overwhelmed or out of control (Berek, 2006)

2.2.2. Menstrual Knowledge Questionnaire (MKQ)

It is a collection of 10 questions to assess dysmenorrheic girls' knowledge about menstrual period. It is designed based on obstetrics and gynecology textbooks by investigators. A correct answer was scored 1 and an incorrect answer or an answer of "I don't know" was scored 0. An overall Content Validity Index (CVI) for MKQ was calculated 0.91 and revealed high content validity. Reliability was assessed using Cronbach's alpha and the coefficient alpha was 0.82.

2.2.3. Menstrual Attitude Questionnaire (MAQ)

The original questionnaire was developed by Brooks and Ruble (1980) with 35 items (Brooks, 1980). In this study, based upon the item analysis, one item of the last subscale was deleted. (Others should not be critical of a woman who is easily upset before or during her menstrual period). Therefore, the Iranian version of this questionnaire contained 34 statements that were rated on a seven-point scale (strongly disagree = 1, strongly agree = 7). Cronbach's alpha coefficient was calculated to determine the internal consistency of each subscale in terms of menstruation as a debilitating event (r=0.72), menstruation as a bothersome event (r=0.79), menstruation as a natural event (r=0.80), anticipation and prediction of the onset of menstruation (r=0.61), denial of any effect of menstruation (r=0.66), and the coefficient alpha for the 34 items as a whole was 0.71. CVI for the Menstrual Attitude Questionnaire was calculated 0.88.

2.3. Data analysis

Sample size was determined based on the findings from a pilot study. In addition, we estimated that a 20 percent decrease would occur in each groups. This calculation showed that a sample of 65 girls with dysmenorrhea per group would be needed to detect a difference between the groups with regard to menstrual pain, with a power of 80 percent and type I error (alpha) of 5 percent. One-way ANOVA and Kruskal-Wallis were used to compare the continuous numerical variables. For categorical variables, the chi-square and Fisher exact tests were used.

2.4. Ethical and confidentiality considerations

The study received ethical approval from the committee for research on human subjects of Mashhad University of Medical Sciences. All subjects gave written consent for participation. They received a thorough explanation of the purpose and procedures of the study and informed that they could withdraw from the study any time without any consequences.

3. Results

The recruited sample comprised a total of 209 dysmenorrheic girls. Among them, 14 clients were selected as peer educators and 30 clients were excluded from the study, constituting a drop-out rate of 15.3 percent. The demographic and menstrual characteristics of the subjects (n=165) in the three groups are shown in Table 1. The distribution of all recorded characteristics didn't indicate a significant difference among the groups. The mean of menstrual knowledge score in the second menstrual period after intervention was significantly higher than baseline in both groups compared to the control group (Table 2).There was no significant difference between the peer-led versus the health-provider-led self-care education group in increasing the menstrual knowledge score after intervention (P=0.128) although it was higher in the health-provider-led self-care education group (Table 2).

Table 1. The demographic and menstrual characteristics of the participants.

	Peer-led education group (n=54)	Health provider-led education group (n=50)	Control group (n=61)	
	Mean (SD) or %	Mean (SD) or %	Mean (SD) or %	P
Age (years)	21.7 (1.5)	21.4 (1.4)	21.7 (1.1)	0.402
BMI (kg/m^2)	22.5 (1.9)	22.6 (1.8)	22.7 (2.0)	0.836
Menstrual cycle length (day)	30.1 (2.2)	29.1 (1.7)	29.7 (2.0)	0.057
Bleeding cycle length (day)	6.0 (1.0)	6.1 (1.1)	6.3 (0.9)	0.302
Age of menarche (years)	13.4 (1.0)	13.7 (1.1)	13.3 (1.4)	0.315
Age of onset of menstrual pain (years)	14.4 (1.5)	15.1 (1.4)	14.8 (2.2)	0.143
Severity of dysmenorrhea (Based on VerbalMultidimensional Scoring System)				
Degree 1	9.3	10.0	6.6	
Degree 2	70.4	80.0	73.8	0.586
Degree 3	20.4	10.0	19.7	
Duration of menstrual pain (hours)	35.8 (9.1)	37.6 (4.8)	37.1 (6.2)	0.632
Frequency of cycles combined with dysmenorrhea during the past six months	4.8 (1.0)	5.2 (1.0)	5.2 (0.8)	0.053

Table 2. Comparison of menstrual knowledge and attitude in peer-led education, health-provider-led education, and control groups

	Peer-led education group (n=54)	Health-provider-led education group (n=50)	Control group (n=61)	
	Mean (SD) or %	Mean (SD) or %	Mean (SD) or %	P
Menstrual knowledge				
Baseline cycle	4.1 (1.9)	3.6 (1.4)	4.1 (1.8)	0.185
The second cycle after intervention	8.8 (1.5)	9.2 (1.3)	4.9 (2.3)	0.000
p-Value	0.000	0.000	0.676	
Menstrual attitude				
Menstruation as a debilitating event				
Baseline cycle	56.6 (8.4)	56.9 (7.5)	55.6 (10.8)	0.732
The second cycle after intervention	40.2 (13.1)	48.3 (9.1)	59.3 (12.1)	0.019
p-Value	0.009	0.035	0.081	
Menstruation as a bothersome event				
Baseline cycle	26.1 (7.9)	24.4 (7.5)	24.3 (7.1)	0.350
The second cycle after intervention	19.6 (7.3)	22.1 (8.8)	23.8 (6.4)	0.047
p-Value	0.000	0.071	0.824	
Menstruation as a natural event				
Baseline cycle	27.2 (4.6)	27.6 (3.6)	26.1 (4.2)	0.152
The second cycle after intervention	29.1 (3.2)	30.1 (2.4)	26.9 (5.5)	0.076
p-Value	0.821	0.619	0.988	
Prediction of the onset of menstruation				
Baseline cycle	27.4 (4.6)	25.9 (3.8)	26.5 (5.1)	0.235
The second cycle after intervention	26.8 (5.9)	27.3 (4.2)	25.8 (6.7)	0.118
p-Value	0.456	0.320	0.189	
Denial of any effect of menstruation				
Baseline cycle	24.2 (5.3)	25.4 (5.0)	23.4 (5.4)	0.155
The second cycle after intervention	28.6 (3.8)	30.1 (5.0)	25.4 (6.1)	0.215
p-Value	0.123	0.099	0.415	

Table 3. Comparison of menstrual cycle's characteristics in peer-led education, health-provider-led education, and control groups

	Peer-led education group (n=54)	Health provider-led education group (n=50)	Control group (n=61)	
	Mean (SD) or %	Mean (SD) or %	Mean (SD) or %	p-Value
Severity of dysmenorrhea				
Baseline cycle	5.0 (1.9)	4.7 (1.8)	4.3 (2.2)	0.164
The first cycle after intervention	4.3 (2.0)	4.0 (1.8)	5.4 (2.2)	0.001
The second cycle after intervention	3.2 (1.2)	2.7 (1.3)	4.5 (2.0)	0.000
Menstrual blood loss (cc)				
Baseline cycle	95.2 (58.4)	83.8 (46.7)	84.1 (53.3)	0.457
The first cycle after intervention	96.1 (41.2)	90.6 (43.8)	88.9 (50.2)	0.312
The second cycle after intervention	90.0 (36.5)	94.1 (41.9)	86.2 (47.5)	0.503
More than five symptoms of PMS				
Baseline cycle	53.7	62.0	68.9	0.248
The first cycle after intervention	49.9	58.3	65.7	0.201
The second cycle after intervention	40.1	55.6	70.3	0.002
Use of painkiller in the first day of menstrual cycle				
Baseline cycle	65.9	68.3	54.2	0.288
The first cycle after intervention	60.2	59.9	58.0	0.313
The second cycle after intervention	57.5	52.2	50.6	0.409
Menstrual blood loss (cc)				
Baseline cycle	95.2 (58.4)	83.8 (46.7)	84.1 (53.3)	0.457

The mean of menstrual attitude score in two subscales of the Menstrual Attitude Questionnaire was significantly lower in the peer-led self-care education group compared with the health- provider-led group (Table 2). Both of the subscales "menstruation as a debilitating event" and "menstruation as a bothersome event" had negative concept and their decreasing scores confirmed the positive effect of the peer education program in this study.

Table 3 shows the comparison of the menstrual cycle's characteristics including severity of dysmenorrhea, menstrual blood loss, symptoms of PMS, and pattern of painkiller use among the three groups before and one and two menstrual period after intervention. There was no significant difference between study groups in the mean scores of severity of dysmenorrhea in the baseline menstrual period (p=0.164). But significant differences were discovered in the mean of severity of dysmenorrhea in the first menstrual period after intervention (p=0.001). The results of the Tukey test showed that there was significant difference in the mean of severity of dysmenorrhea between peer-led self-care education and control groups (4.3±2.0 VS 5.4±2.2, p=0.015) and health-provider-led self-care education and control groups (4.0±1.8 VS 5.4±2.2, p= 0.001) but there was no significant difference in the mean of severity of dysmenorrhea between peer-led self-care education and health-provider-led self-care education groups (4.3±2.0 VS 4.0±1.8, p= 0.715). Moreover, the results in the second menstrual period after intervention were like the results in the first one. There was significant difference between study groups in the mean of severity of dysmenorrhea in the second menstrual period (p=0.000). Based on the results of the Tukey test, there was a significant difference in the mean of severity of dysmenorrhea between peer-led self- care education and control groups (3.2±1.2 VS 4.5±2.0, p=0.000) and health-provider-led self care education and control groups (2.7±1.3 VS 4.5±2.0, p= 0.000) but there was no significant difference in the mean of severity of dysmenorrhea between peer-led self-care education and health-provider-led self-care education groups (3.2±1.2 VS 2.7±1.3, p= 0.331) (Table 3).

In the second menstrual period after intervention, 40.1 percent of the peer-led self-care education group, 55.6 percent of the health-provider-led self-care education group and 70.3 percent of the

control group had experienced more than five symptoms of PMS. There was significant difference between education groups and control, but there was no significant difference in the percentage of those who had experienced more than five symptoms of PMS between peer-led self-care education and health-provider-led self-care education groups (p=0.575). There was no significant difference between study groups in the mean of menstrual blood loss (p=0.457, p=0.312, p=0.503) and use of painkillers in the first day of menstrual cycles (p=0.288, p=0.313, p=0.409) before and after intervention.

4. Discussions

The results of the present study demonstrated that peer-led self-care education is as effective as the health-provider-led education at promoting health behaviors and reducing pain in primary dysmenorrheic girls. Crotty et al. (2009) investigated the impact of peer-led self-care education on the reduction of pain in those afflicted with arthritis (Crotty, 2009). They didn't report any significant difference between the control and the intervention groups in terms of the degree of pain before and after the intervention stage. In Crotty's research, the peer group consisted of patients afflicted with osteoarthritis who experienced severe pain once entering the research phase. In the present study, the peer group included primary dysmenorrheic girls who had mostly experienced an average degree of pain once entering the research. Therefore, it can be concluded that in any research that aims to assess the impact of peer-led self-care education on the severity of pain, sufficient attention should be paid to the participants' degree of pain upon entering the program.

The other factor that could have led to the success of peer-led self-care education in this study compared to Crotty's is the setting that was chosen here, i.e. the dormitory life-style. Such an environment pulls members closer together and reinforces the sympathetic behavior among girls who suffer from dysmenorrhea. The same factor leads to a better and faster knowledge transfer among the peer-group members. Proponents of peer-led self-care education believe in the necessity of good quality peer education. The peer needs to be given a detailed protocol so that they manage to best play their role as a peer educator. In the present study, peers were instructed by taking part in workshops exclusively for this purpose. In designing the workshop program, use was made of the book "Training of Trainers Manual" published by UNFPA to prepare peers for adopting the educator's role (UNFPA, 2006). Such instruction appeared to be very effective in motivating the peers and creating a feeling of responsibility. Besides that, we provided the peer educators with the instructional content of self-care behaviors related to dysmenorrhea based on Orem's theory. Therefore, it can be concluded that emphasizing the peer educator's role and enabling them to take responsibility of such a role might be one reason for success of the peer education program.

Although the peers' previous experience has been recognized as one of the most influential factors in carrying out their roles (UNODC, 2010), in this study none of the participants were experienced in any instructional programs. Therefore, the present researcher has used other factors to select the peer participants. Some of the issues considered in selecting the peer educators in this study include: cooperation of peer group members in selecting the educator, taking into account the members' interest in adopting the peer educator's role, preparing a greater number of peer educators, followed by selection based on the highest degree of preparation.

Lorig et al. (2009) investigated the effectiveness of a community-based diabetes self-management program (Lorig, 2009). They concluded that people with diabetes could benefit from a peer-led diabetes program. The finding of their research revealed that the peer-led program reduced depression, symptoms of hypoglycemia, and communication with physicians. Moreover, it increased healthy eating, patient activation, and self-efficacy in these patients.

In the present study, part of the self-care education instructional program consisted of controlling the external factors affecting dysmenorrhea, such as the nutrition pattern and activity. Therefore, considering the results of Lorig's study based on improving healthy eating and patient activation, it seems that these factors are positively effective on peer-led self-care education in the present research.

Attention to forming small peer groups and peers' participation in selecting the group members are introduced as effective factors in the success of the peer education program (Fhi, 2010). These factors received adequate attention in the present paper. Having compared the present study with the other related research, we can summarize the effective factors in peer-led self-care education success in the three following categories: factors related to the peer group, peer educator, and designing the peer education program.

Some of the limitations of this research are presented as follows:

1- Impossibility of pelvic examination to diagnose the primary dysmenorrhea due to the virginity of the subjects.

2- Obtaining knowledge of self-care through sources other than the instructional program during the research.
3- Probability of diffusion of information among the research units in the three groups understudied.

Considering the benefits of peer education and the same effect of peer-led self-care education as compared with health-provider-led education on the reduction of pain in primary dysmenorrheic girls, health education systems can use peer education to promote self-care behaviours among primary dysmenorrheic girls. In addition, the findings of this study can be used as a basis for further research in recognizing the effect of peer-led self-care education programs on other health-related subjects.

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Purification, Characterization and Antitumor Activity of L-asparaginase from Chicken liver

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Abstract: Abstract: The L-asparaginase (E.C.3.5.1.1) produced by chicken liver was isolated and characterized. Different purification steps (including ammonium sulphate fractionation followed by separation on Sephadex G-100 gel filtration and Sephadex G-200 gel filtration) were applied to crude filtrate to obtain a pure enzyme preparation. The enzyme was purified 128.5 ± 0.5 fold and showed a final specific activity of 158.11 ± 5.0 U/mg with a 17.1 ± 8.6 % yield. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of the purified enzyme revealed it was one peptide chain with M_r of 33 kDa while by gel filtration appears to be 36 kDa. The enzyme was very specific for L-asparagine and doesn't hydrolyze L-glutamine. A Lineweaver-Burk analysis showed a K_m value of 1.66 mM toward L-asparagine as substrate and V_{max} of 34.47 U. The enzyme showed maximum activity at pH 9.5 when incubated at 60°C for 20 min. The amino acids composition of the purified enzyme was also determined. Antitumor activity was investigated. The enzyme inhibited the growth of the two human cell lines including hepatocellular carcinoma (Hep-G2) and colon carcinoma (Hct-116) with IC_{50} value of 8.38µg/ml and 4.67µg/ml, respectively. While IC_{50} was greater than 10µg/ well for MCF7 (breast carcinoma) cell line.
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Keywords: Chicken liver- gel filtration-purification-amino acid composition- human cancer cell line- antitumor activity.

1. Introduction:

L-asparaginase amidohydrolase (E.C. 3.5.1.1) is an enzyme which catalyzes the hydrolysis of L-asparagine into L-aspartate and ammonia according to the following equation: L-asparagine +H_2O------->L-aspartate+ ammonia.

It is widely distributed in nature, not only in animal organs such as liver of guinea pig, placenta, kidney and intestine of beef and horse (Prista and Kyridio, 2001), but also in microorganisms such as *Escherichia coli*, *Thermus thermophius* , *Erwinia caratovora* (Kotizia and Labrou, 2005; Michalska, *et al.*, 2006; Kotzia and Labrou, 2007; Verma *et al*., 2007 and Tabandeh and Aminlari, 2009) and also in plants such as soy beans, *Oryza sativa, Hordenum vulgare* and *Lupinus* species (Borek and Jaskolski, 2001).

Interest in this enzyme arose a few decades ago when it was discovered that the antilymphoma activity of whole guinea pig serum was result of the enzyme L-asparaginase (Prista and Kyridio, 2001). The anti-leukemic effect of L-asparaginase is a result of rapid and complete depletion of the circulating pool of L-asparagine. As in a great number of patients with lymphoblastic leukemia, the malignant cells depend on exogenous source of L-asparagine to be able to survive, mean while, the normal cells are able to synthesize L-asparagine (Narta *et al*., 2007). The discovery of new L-asparaginase serologically different but having a similar therapeutic effect is highly desired, (Moharam *et al*., 2010). The important application of the L-asparaginase enzyme is in the treatment of acute lymphoblastic leukemia (mainly in children), Hodgkin disease, acute myelocytic leukemia, chronic lymphocytic leukemia, lymphosarcoma treatment, reticulosarcoma and melanosarcoma (Tabandeh and Aminlari, 2009 and Sunitha *et al*., 2010).

Little work has been carried out on L-asparaginase from chicken liver. The present paper is devoted to the purification of an asparaginase from chicken liver and to a comparative study of some of its biochemical and biological properties.

2. Materials and methods

Chemicals:

Anhydrous L-asparagine , trichloroacetic acid, Nessler reagent chemicals (HgI_2, KI and sodium hydroxide, molecular weight markers for gel filtration, all resins and reagents for electrophoresis were obtained from Sigma chemical CO. (St Louis, Mo). Sephadex G-100, Sephadex G-200 for chromatography and molecular weight markers for SDS-polyacrylamide gel electrophoresis were obtained from Pharmacia Fine Chemicals (Sweden). Buffers were prepared according to the method of Gomori (1955), and the final pH values were checked on Hanna pH meter. All the other chemicals were analytical grade.

Animals:

The screening was carried out with different animal's serum and organs such as liver, lung, kidney, testis, ovaries, heart, pancreas, spleen and brain from mouse, rabbits, chicken, buffalos and rats. Where the chickens and rabbits were brought from markets. Buffalos were brought from EL-Bassatein's slaughter house and finally rats and mice were brought from animal's house in National Research Center, Giza.

The enzyme activities of homogenates of organs and supernatants of serum from different species (crude enzyme) with different buffers and molarities that are commercially available in a large quantity were studied in comparison with liver homogenates of laboratory animals.

All experiments were carried out with chicken livers. They were obtained from animals of random breed and sex, maintained from markets, liver was kept frozen at - 40°C.

L-asparaginase assay:

The enzyme activity was assayed according to Wriston (1970) method. The reaction mixture contained 0.9 ml of 0.01mole L-asparagine preparation in 0.05 mole sodium borate buffer, pH 8.5 and adequate amount of L-asparaginase was incubated for 20 min at 37°C. The reaction mixture was centrifuged at 6000 xg for 10 min and the ammonia released in the supernatant was determined by Nesslerization reaction. In brief, to 0.5 ml of supernatant, 1.75 ml distilled water, 0.25 ml of Nessler reagent was added. After 10 min; absorbance at 480 nm were read with appropriate control.

One enzyme unit (U) is defined as the amount of enzyme that librates one µmole of ammonia per min at 37°C. Standard curve of ammonium sulphate was used for calculating ammonia concentration. The activity values of samples present in the paper were average values of three repeated measurements. Where the specific activity is defined as the units of L-asparaginase per milligram protein (Bansal *et al.*, 2010).

Protein determination:

The total protein contents of the samples were determined according to the method described by Lowry *et al.* (1951) using bovine serum albumin as standard.

Purification of enzyme:

The purification was carried out at 4°C on the crude enzyme by:

1. Ammonium sulphate precipitation:

Certain volume of the prepared crude enzyme was treated with different concentration of ammonium sulphate (20-60%). The mixture was left at 4°C over night, follwed by centrifugation at 13000 r.p.m for 15 mins at 4°C. The resulting precipitates were dissolved in appropriate amount of distilled water and dialyzed exhaustively against distilled water for 2 days at 4°C to get ride of the excess of ammonium sulphate.

2-Sephadex G-100 gel filtration:

The dialyzed ammonium sulphate fraction was applied to Sephadex G-100 column (1.2 x55 cm) was pre-equilibrated with 0.01 M sodium borate buffer pH 8.5 at a flow rate of 20 ml/h. The fraction were collected and examined for enzyme activity and protein content.

3-Sephadex G-200 gel filtration:

The fraction from Sephadex G-100 with high L-asparaginase activity was loaded onto the pre-equilibrated Sephadex G-200 column (1.2 x 55 cm) with 0.01 M sodium borate buffer, pH 8.5 at a flow rate of 16 ml/h. The fractions were collected and examined for enzyme activity and protein content.

Native-PAGE: a slab gel electrophoresis was carried out using a 15% poly-acrylamide gel (pH 6.2). After electrophoresis in a tris-glycine buffer (pH 8.3) at 200V for 7h at 70°C, the proteins in the gel were stained with coomassie brilliant blue R-250 and destined (EL-Gamal et al., 2001).

Molecular weight determination by:

1- SDS-PAGE: was performed following the method of (Laemmli, 1970) with separating acrylamide gel 12.5% (wt/vol) and stacking gel 3% (wt/vol) containing 0.1% (wt/vol) SDS. The log molecular weight of different standard molecular weight marker proteins of 66 kDa (bovine serum albumin), 45 kDa (egg albumin), 36 kDa (glyceraldehyde-3-p-dehydrogenase), 29 kDa (carbonic dehydrogenase bovine), 24 kDa (trypsinogn bovine pancrease), 20 kDa (trypsin inhibitor soybean) and 14.2 kDa (∞-lactoalbumin bovine milk) were plotted against their relative mobility in the gel, and from the plot the molecular weight of the protein was calculated. The gel was stained with Coomassie brilliant blue R-250.

2-Gel filtration: the molecular weight of the purified enzyme was estimated by gel filtration chromatography through a column (1.2 x 40 cm) of Sephadex G-200 as described by Andrew (1964), pre-equilibrated with 0.01 M sodium borate buffer pH 8.5. The column was calibrated with standard molecular weight marker proteins as: 66 kDa (bovine serum albumin), 33 kDa (trypsin from porcine pancrease), 29 kDa (carbonic anhydrase), 20.1 kDa (trypsin inhibitor), and 14.2 kDa (lyzozyme).

Amino acid composition:

Purified L- asparaginase was dissolved in one ml of dilution buffer/Eppendorf-Germany, and then

injected into full automated amino acid analyzer, eppendorf LC 3000**.**

The conditions were estimated to be flow rate 0.2 ml/min**,** pressure of buffer form 0 to 50 bar, pressure of reagent to 0-150 bar and reaction temperature (123°C).

Antitumor activity:

Potential cytotoxic activity against some tumor cell line was performed in the National Cancer Institute using method of Skehan *et al.* (1990).

Prepared L-asparaginase partially pure (ammonium sulphate) and pure enzymes were lyophilized. One milligram of each lypholized powders were dissolved in 0.1 ml of DMSO and the volume completed to one ml with distilled water.

Cells were plated in (10^4 cells/well) for 24 h before treatment with the dried L-asparaginase to allow attachment of cell to the plate.

Different concentrations of the compound under test (0, 1, 2.5,5,10 µg/ml) were tested. Triplicate tested were prepared for each individual dose.

Monolayer cells were incubated for 48 h at 37°C in atmosphere of 5% CO_2, after 48 h cells were fixed, washed stained with sulforhodamine-B stain. Excess stain was washed with acetic acid and attached stain was recovered with tris EDTA buffer. Color intensity was measured in an ELISA reader.

The relation between surviving fraction and the drug concentration is plotted to get the survival curve of each tumor cell line for the specified enzyme.

The effective dose required to inhibit cell growth by 50% (IC_{50} µg/ml) was determined. Doxorubicin was used as positive control.

Dried L-asparaginase was tested for the following tumor cell lines at concentration between 1.0-10.0 µg/ml:

Hepatocellular carcinoma cell line.

Breast carcinoma cell line.

Colon carcinoma cell line.

3. Results and Discussion.

L-asparaginase of the liver of various species:

The enzyme activities of homogenates of liver from different species that are commercially available in large quantities were studied in comparison with liver homogenates of laboratory animals and liver of buffalo. The results are shown in table (1). The result encouraged the use of chicken liver for the further studies as it has a higher L-asparaginase activity, 8.66 U/gm of liver than the other livers with specific activity, 0.648 U /mg at optimum assay condition.

Preparations of the enzyme from chicken liver:

Liver was homogenized with sand and extracted in twice their volume of 0.01 M sodium phosphate buffer, pH 7.4 containing 0.1 M potassium chloride. They were incubated separately with L-asparagine dissolved in 0.05 M sodium borate buffer, pH 8.5 at 37°C for 30 mins.

Purification of L-asparaginase enzyme from chicken liver:

Many steps commonly employed for enzyme purification were inapplicable. The enzyme activity (L-asparaginase) was destroyed by organic solvents (acetone precipitation). DEAE-cellulose column could not be employed successfully owing to the low stability of the enzyme at salt concentrations.

The partial purification of the L-asparaginase crude extract that was affected by the ammonium sulphate (20-60%) saturation showed that most of the enzyme activity was preserved in the precipitate. The total protein concentration was decreased from 238 ± 1.9 to 64 ± 0.46 mg in ammonium sulphate precipitate with 55.3±1.2 % yield.

Fig. (1) shows the elution profile of purification of the ammonium sulphate fraction (20-60 %) on Sephadex G-100 column. This fraction contained wide peak with L-asparaginase activity with specific activity 18.2 ± 2.2 U/mg.

The elution profile of the most active fraction, collected from Sephadex G-100 on Sephadex G-200 column was illustrated in fig (2).

Although this fraction contained three different protein peaks, only one peak showed L-asparaginase. A sharp distinctive peak of L-asparaginase activity which fits with only one protein peak was obtained (tubes number 12 and 13) as shown in fig.(2). The various steps of the purification procedure finally adopted by a relative simple method and are shown with summarizing data in table (2). The activity values were average values of nine repeated purification batches.

Thus, purification of L-asparaginase to homogeneity from chicken liver was achieved by simple steps with final yield 17.1 ± 8.6 %, a purification fold 128.5 ± 0.5 and a specific activity of 158.11 ± 5.0 U/mg protein.

Table (1): L-asparaginase activity in the livers of various species:

Species	Wet weight/Volume (gm/ml)	L-asparaginase Activity (U/ml)	Total units	Total L-asparainase activities (U/gm) of liver	Protein conc. (mg/ml)	Specific activity (U/mg)
Mouse	2.19/7	0.0	0.0	0.0	7.26	0.0
Rabbit	5.04/8.2	1.478	12.12	2.40	5.240	0.28
Chicken	4.42/6	6.384	38.30	8.66	9.85	0.648
Buffalo	9.50/18.9	2.497	47.20	4.96	11.10	0.224
Rat (female)	11/22	2.077	45.69	6.06	-	-
Rat (male)	7.39/20.1	1.182	23.76	3.21	7.990	0.147

Note: 0.05 M sodium borate buffer pH 8.5 at 37°C for 10 min.

Table (2): Purification profile of L-asparaginase from fresh chicken liver (10 g).

Purification steps	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification fold	Yield (%)
Crude extract	284±2.4	238 ±1.9	1.23±0.1	1.0	100
Ammonium sulphate fraction (20-60%)	158±1.0	64±0.46	2.47± 0.3	2.0 ± 0.3	55.3 ±1.2
Gel filtration on Sephadex G-100	100 ±7.0	5.53 ±0.09	18.2 ±2.2	14.8 ±0.23	34.8 ±2.0
Gel filtration on Sephadex G-200	48 ± 3.0	0.305 ±0.02	158.11 ±5.0	128.5 ±0.5	17.1± 8.6

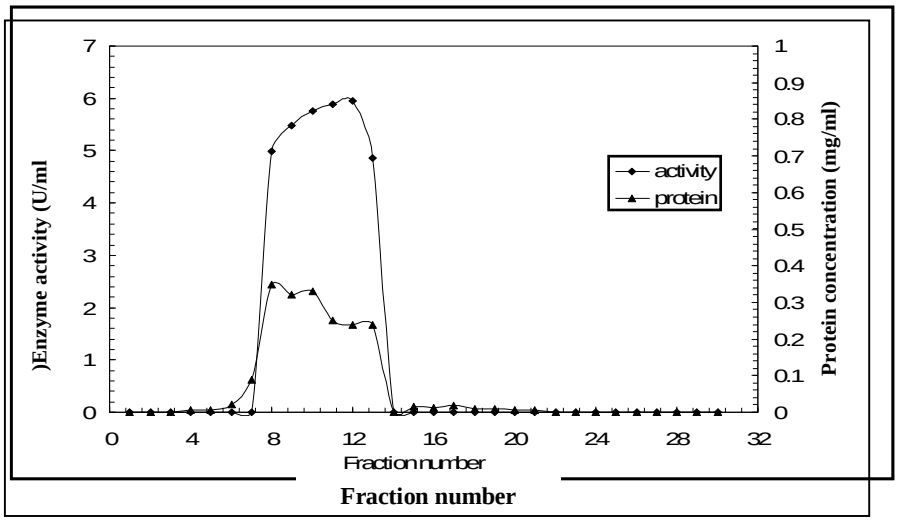

Fig. (1): Elution profile of L-asparaginase on Sephadex G-100 column.

The dialyzed ammonium sulphate precipitate (20-60%) was chromatographed on Sephadex G-100 in (1.3x 55 cm) column. The column was equilibrated and eluted with 0.01 M borate buffer pH 8.5. The fractions were assayed for L-asparaginase activity and protein content.

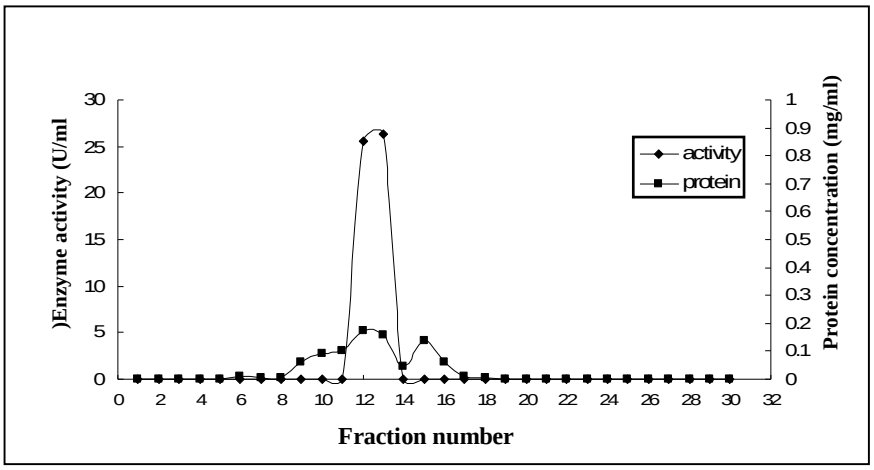

Fig. (2): Elution profile of L-asparaginase on Sephadex G-200 column.

The most active collected fraction from Sephadex G-100 was applied to Sephadex G-200 (1.2 x 55 cm). The fractions were assayed for the enzyme activity and protein content.

Molecular weight of L-asparaginase (Figure 3):

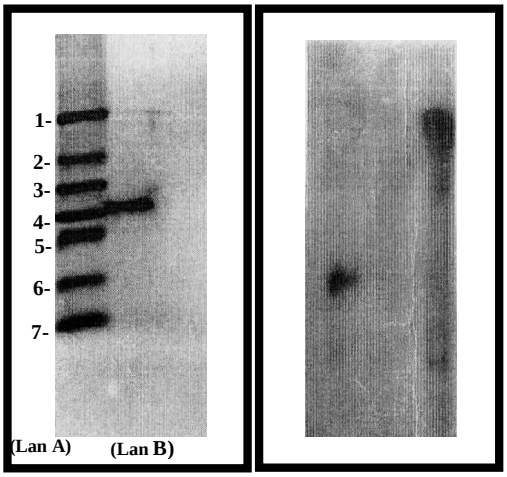

Fig. (3a&b): Native and PAGE –SDS of L-asparaginase from chicken liver.

Lane A: Included the following standard proteins:

1- Bovine serum albumin (66,000).
2- Egg albumin (45,000),
3- Glyceraldehyde-3-p-dehydrogenase (36,000),
4- Carbonic dehydrogenase bovine (29,000),
5- Trypsinogn bovine pancrease (24,000),
6- Trypsin inhibitor soybean (20,000) and ∞-lactoalbumin bovine milk (14,200)
7- Lane B: Purified enzyme (5µg).

Native-PAGE of the purified enzyme preparation from Sephadex G-200 column was performed to get basic information about the purity of the L-asparaginase. It was revealed only one distinctive band as shown in fig. (3a).

SDS–PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis), was performed with the purified enzyme. The result revealed to no detectable contamination and a single distinct band was observed with molecular weight of about 33 kDa, fig (3b).

Determination of molecular weight of L-asparaginase by gel filtration (Sephadex G-200): The molecular weight of the purified enzyme is found to be 36 kDa. By using different standard proteins with known molecular weights, it was found that the apparent molecular weight of chicken liver L-asparaginase preparation was 36 kDa, fig. (4).

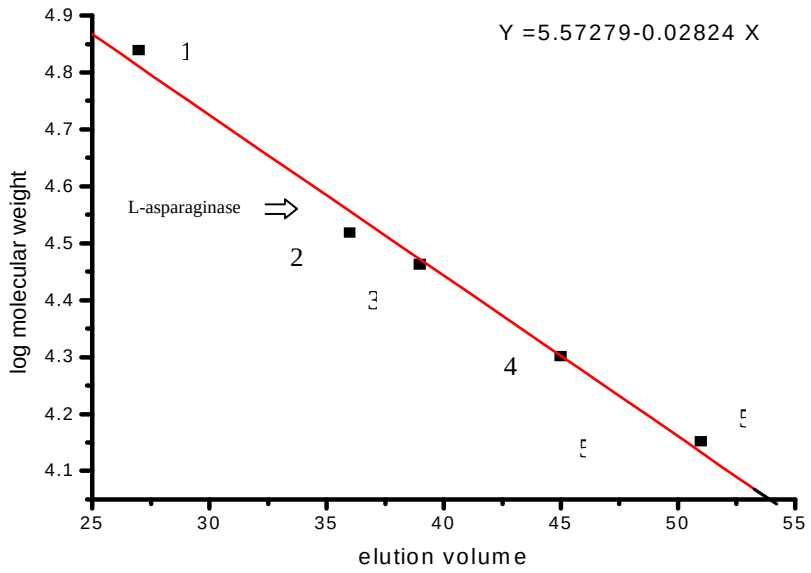

Fig. (4): Determination of the molecular weight of the purified L-asparaginase by gel filtration on Sephadex G-200 column (1.2 X 40 cm). 1) Bovine serum albumin (66,000) 2) trypsin from porcine (33,000) 3) carbonic anhydrase from bovine erythrocytes (29,000), 4) trypsin from soybean inhibitor(20,000), 5) lysozyme (14,200).

In this respect, the enzyme was approximately similar to that obtained from *Pseudomonas stutzeir* MB-405, *Thermus thermophilus* and *Eshirichia coli* with M_r range from 33-34 kDa, (Manna *et al.*, 1995; Prista &Kyridio, 2001 and Soares *et al*., 2002). While the enzyme was lower than that obtained from *Pisum sativum* (Sieciechowicz *et al.*, 1989). M_r of L-asparaginases isolated from *Pseudomonas aeruginosa* 50071 and *Chlamydomonas* sp were approximately 160 kDa, (EL-Bessoumy *et al*., 2004 and Dhevagi and Poorani, 2006).

Physicochemical properties of the purified L-asparaginase:

The pH influence on the L-asparaginase activity was studied using a 0.05 M borate buffer of different pH values ranging from 4 to 11.5. The enzyme activity gradually increased until pH 7.5 and remains high active over a wide range of pHs' from 7.5 to11 and at which the maximum activity was observed, fig (5). At higher pH than pH 11, the enzyme activity was decreased to 33.3%. A similar pHs' values were obtained from Guinea pig serum, Pseudomonas stutzeir MB-405 and from Helicobacter pylori (Tower et al., 1963; Manna et al., 1995 and Cappelletti et al., 2008).

The effect of the incubation time on L-asparaginase activity was studied in the ranges of 5 to 180 min, (Fig.6). L-asparaginase activities increased as the incubation time increased. The activity ran at maximum for 30 minutes and still maximum for 90 min. After 90 min, it decreased as the time increased.

The reaction rate of L-asparaginase was measured at various temperatures from 40 to 75°C, (Fig.7). It appears that L-asparaginase optimally deamidated at 60°C. At higher temperature than 60°C the reaction rate

declined to 77.2% of activity 75°C. When the enzyme was exposed in absence of the substrate to 30°C up to 45°C for 60 min, then their activities were measured, as described before the activity was about 52 % increased, (Fig.8). Beyond this temperature the enzyme becomes increasingly unstable. Similar results were recorded for asparaginase from *Pencillium politans NRC 510* (Tower *et al*., 1963 and Ali and EL-Sayed, 2006). They were proved that *Thermus thermophilus* and Guinea pig serum L-asparaginas, are quite stable and linear even at 70°C or 77°C. On the other hand, L-asparaginase from *Erwinia* sp had a maximum activity at 35°C (Borkotaky and Bezbaruah, 2002).

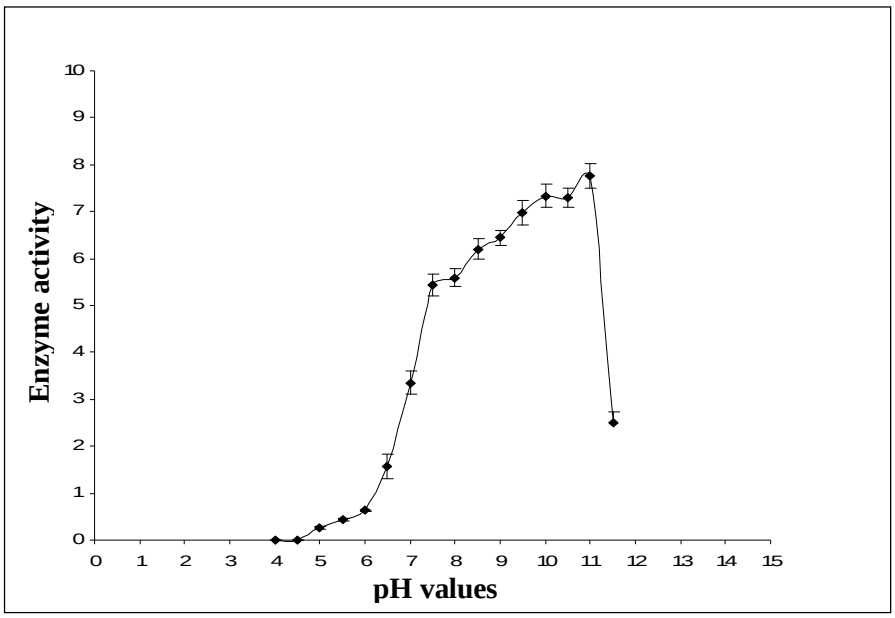

Fig. (5): Effect of pH on L-asparaginase activity.

The purified L-asparaginase was very specific for L-asparagine and low for DL-asparagine (22.5%) and did not hydrolyzed L-glutamine. L-asparaginase of different microorganism has different substrate affinities and probably plays a different physiological role in the enzyme activity, (EL-Bessoumy *et al*., 2004).

The K_m value of the purified enzyme was determined according to the method of Lineweaver and Burk (1934). A Lineweaver-Burk analysis gave K_m of 1.66 mM toward L-asparagine as substrate and the maximum velocity (V_{max}) of 34.47 U (Fig.9). Higher K_m values (6.6 and 7.0 mM) for L-asparaginase from *Lupinus arboreus* and *Lupinus angustifolius*, respectively has been reported by Chang and Franden (1981). On The other hand, a lower K_m value (0.058 mM) was obtained for L-asparaginase from *Erwinia chrysanthemi* 3937 *(*Kotzia and Labrou, 2007).

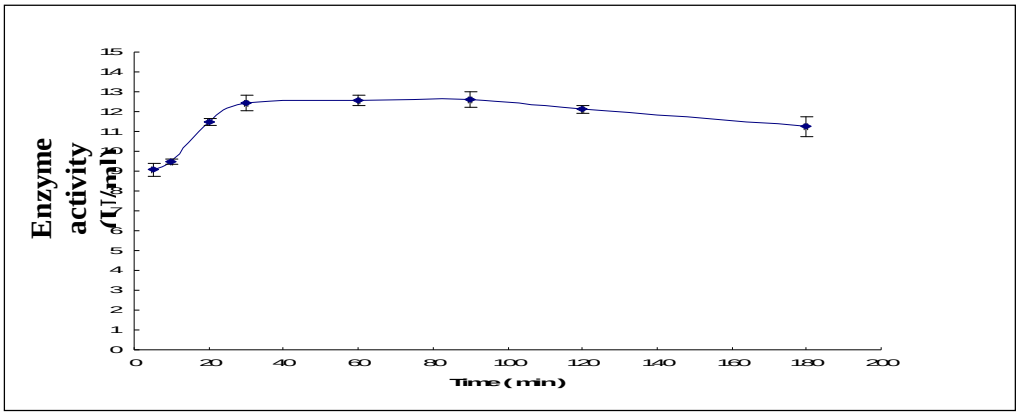

Fig. (6): Effect of time on the pure L-asparaginase activity.

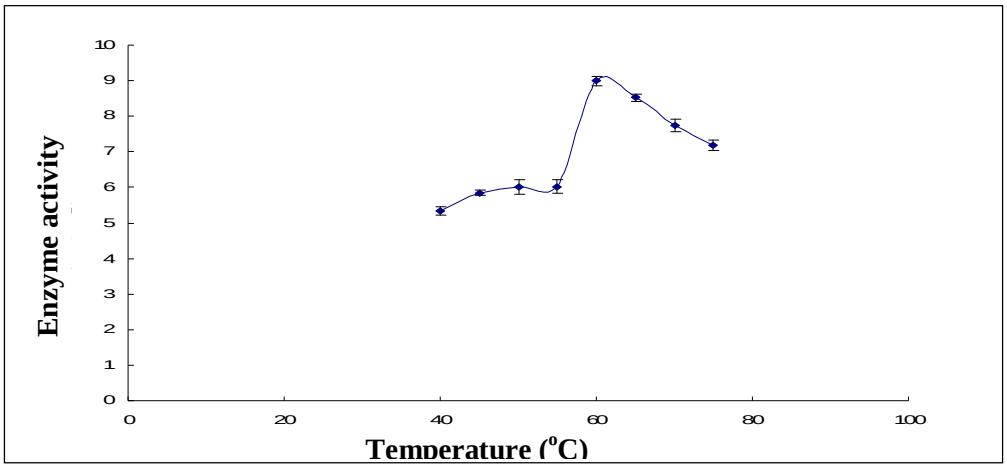

Fig. (7): Effect of temperature on L-asparaginase activity.

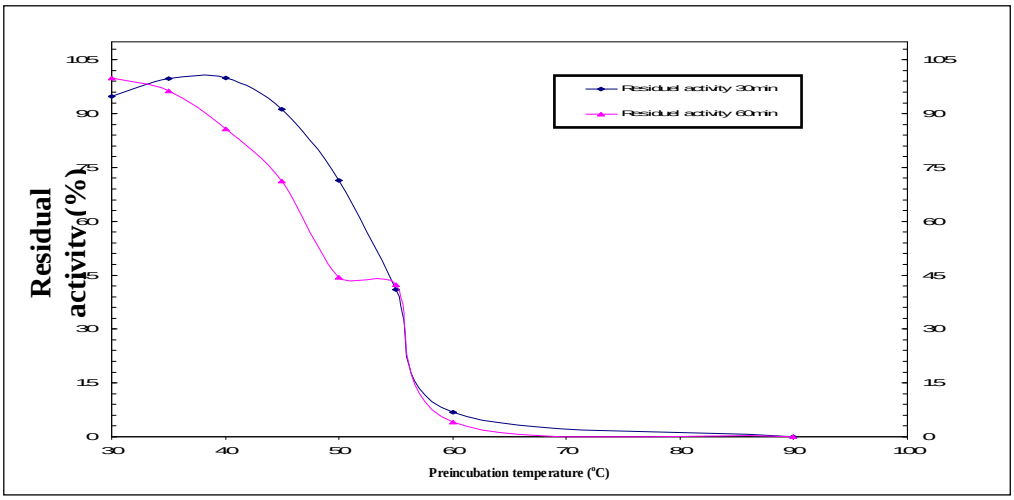

Fig. (8): Thermal stability of L-asparaginase activity.

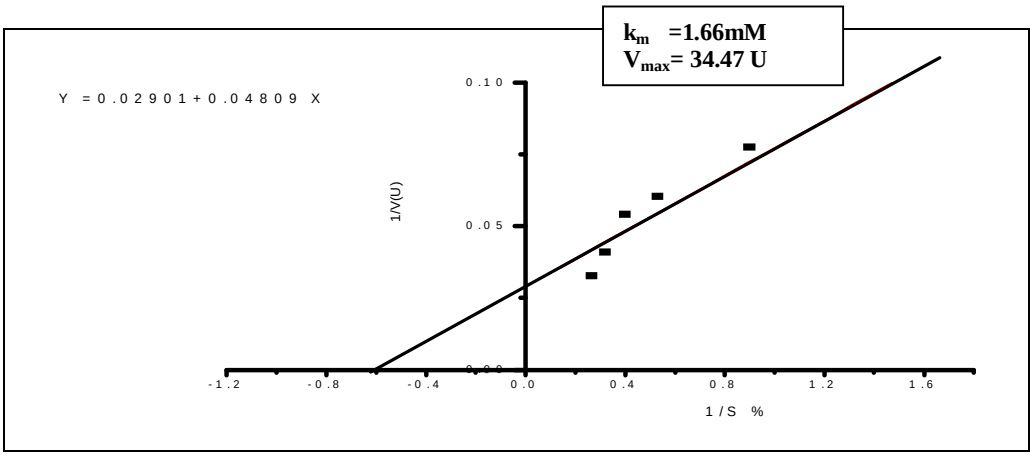

Fig. (9): Lineweaver –Burk plot of L-asparaginase activity using L-asparaginase as substrate.

Amino acid composition:

Table (3) shows the amino acid contents of the purified chicken liver L-asparaginase.

Table (3): Amino acid contents of the purified chicken liver L-asparaginase.

Amino acid concentration (mol%)	Amino acid
12.64	Aspartic acid
2.03	Threonine
2.35	Sereine
6.48	Glutamic acid
2.30	Glycine
4.65	Alanine
7.73	Cystin
0.095	Methionine
2.43	Isoleucine
4.64	Leucine
1.22	Tyrosine
1.71	Phenylalanine
1.39	Histidine
2.78	Lysine
1.86	Arginine
0.39	Proline

The quality of chicken liver L-asparaginase was assessed for its amino acid contents. The purified enzyme was rich in aspartic acid, glutamic acid and cystin. **Qian *et al*. (1996)** reported that aspartic acid protects the active site of *Esherichia coli* L-asparaginase.

Biological properties:

The lypholized L-asparaginase enzyme (partial and pure) was subjected to cytotoxic activity *in vitro* on the cell lines available HEPG2 (hepatic carcinoma), HCT (colon carcinoma) and MCF7 (breast carcinoma) using SRB assay. The growth inhibition data were expressed as percent of control. Results in figs. (10 and 11) shows that no significant differences were observed in the cytotoxicity between the highly purified and partially purified L-asparaginase enzyme against HEPG2 (hepatic carcinoma) cell line (IC_{50} = 8.38 µg /well and 8.91 µg / well respectively). While results showed difference between the cytotoxicity of highly purified and partially purified L-asparaginase enzyme against HCT (colon carcinoma) cell line (IC_{50} = 4.67 µg / Well and 6.44 µg /well respectively).While IC_{50} was greater than 10µg/ well for MCF7 (breast carcinoma) cell line. The sensitivity of MCF, HEPG2 and HCT cells to both asparaginases (partial and pure fraction) appeared to be dose dependent, resulting in significant decrease in viable cells. Treatment of different tumor cancer cell lines with increasing the concentrations of L-asparaginase up to10 µg results in appreciable inhibition of the cell growth.

Cappelletti *et al*. (2008) studied *in vitro* cytotoxicity of a novel L-asparaginase from the pathogenic strain *Helicobacter pylori* CCUG 17874 against different cell lines. They reported that AGS and MKN 28 gastric epithelial cells being the most affected. While in breast cell line used in this investigation do not contain L-asparagine synthetase activity (Prista *et al*., 2001).Therefore, the selective growth inhibition by L-asparaginase of breast cancer cell could be related to the absence of intracellular L-asparagine synthetase activity in this cell.

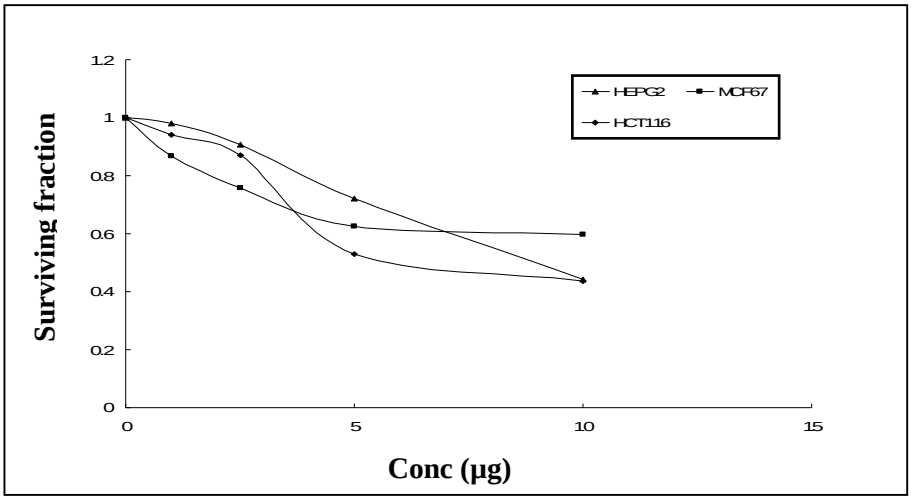

Fig (10): Cytotoxic activity of partially pure L-asparaginase.

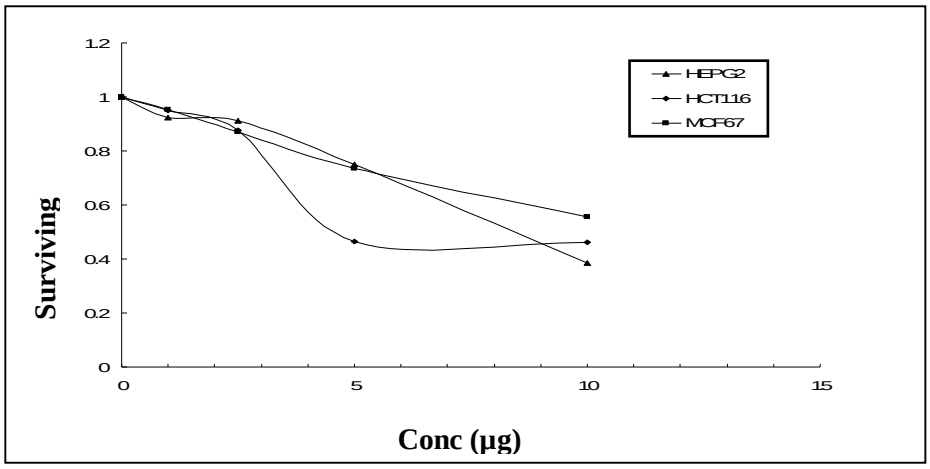

Fig (11): Cytotoxic activity of pure L-asparaginase.

Our results showed that the purified L-asparaginase from chicken liver has got the favorable activity at wide range pH, high temperature, high affinity towards L-asparagine, no glutaminase activity and good heat stability which deserve further investigations on chicken liver L-asparaginase for its proper utilization. Also, the results showed that L-asparaginase has anti-proliferative activity in different cell lines growth *in vitro* (antitumor activity against hepatic and colon carcinoma).

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Nursing Intervention Program for Early Detection and Prevention of Breast Cancer among Working Women

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Abstract: Aim: of the study was to assess the impact of a nursing intervention program leading to health decisions for breast cancer screening among working women with the hypothesis that the intervention will improve women knowledge, modify their attitude, and empower them to take informed health decisions for breast cancer screening. Design: This quasi-experimental design Setting: was conducted in 2 pharmaceutical companies, 2 food processing industries, and a textile factory Sample: a convenience sample 520 women working previous settings, Tools: used for data collection included a self-administered assessment questionnaire assessing knowledge, a health beliefs assessment rating scale, an attitude rating scale, a breast self-examination observation checklist, and a mammography card. A nursing intervention program was designed by the researchers based on the results obtained from the study tools and findings of similar research. Results: The mean age of studied women was 43.2 years, and 56.7% of them had secondary education. Only 5.4% of the women had satisfactory knowledge at the pretest. After program implementation, statistically significant improvements were revealed in women's knowledge about breast cancer and early detection methods, as well as in their related health beliefs and attitudes .Also,73.3% and 72.9% women successfully perform BSE at the post and follow-up phases ($p<0.001$). The practice of mammogram increased from 4.2% at the pre-intervention to 17.7% at the follow-up ($p> 0.001$). The highest practices were among women working in pharmaceutical companies, those with age 45 of older, and those with positive family history of breast cancer. Conclusion: Working women had deficient knowledge, and negative perceptions related to breast cancer and its early detection; their practice of breast self-examination and mammography was very low. The intervention program had a positive effect on women's knowledge, practice health beliefs and attitude. Recommendations: Continuous workplace educational health programs are recommended. With supportive health insurance. Further research studies with broader range of occupational setting are suggested.
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Keywords: Nursing; Intervention; Breast Cancer; Women

1. Introduction:

Breast cancer is a serious disease with potential high morbidity and mortality. It is the commonest malignancy in women. Approximately one million new cases of breast cancer are diagnosed each year worldwide (Nevidjon and Sawers, 2000; Mahmoud, 2002). In Alexandria, Egypt, out of 9,587 female cancer cases registered in the last 10 years by the Alexandria Cancer Registry, 3250 (33%) had breast cancer (Bedwani et al., 2001).

Although breast cancer cannot be prevented, its early detection offers more treatment options, and a great chance of cure. However, it is usually diagnosed at advanced stages and the survival rate is poor. The use of mammography to screen asymptomatic women 40 years of age and over for early detection of breast cancer has been shown to reduce mortality rates by 20-30% (Aziz and George, 2002).

However, although mammography is established as a screening modality for breast cancer, it is out of reach of many socially disadvantaged women in Egypt, and another approach has to be considered for the early detection of breast cancer (Boulos, 2002). It is therefore, important to promote awareness about early diagnosis of breast cancer and to evaluate the role of screening, recognizing that resources are not available to permit the introduction of mass mammography screening. Hence, physical assessment of the breast should be part of periodic health maintenance examinations, and teaching the client to perform monthly breast self-examination were suggested (Altman, 2004). Clinical breast examination is performed not only to evaluate the patient's specific symptoms but also to identify any other abnormalities of the breast or regional lymphatic basin (Mehata, 2004). The American Cancer Society (2006) issued guidelines with instructions on performing breast self-examination.

Decisions about health and health care are made daily at all levels. There is a growing recognition that consumers want to participate in clinical decisions about their health (Gainer, et al, 2003). Taking an active role in achieving and

maintaining good health depends on certain personal factors. These include perceived susceptibility, level of motivation, sense of control, and perceived value of behavior. People are motivated to take action if they feel that a sufficient thereat to their health exists and the consequences of changing the behavior are worthwhile (Alters and Schiff 2000). The Health Belief Model was designed to predict which people would and would not use preventive measures and to suggest interventions that might reduce client reluctance to use health care (Bensley and Fisher, 2003).

Work sites are an important venue for efforts to reduce cancer morbidity and mortality. Through worksites, it is possible to influence the health behaviors of large proportions of the population based on providing educational risk reduction message targeting individual behavior changes, promotion of environmental supports, and use of natural social network structures (Mary et al, 2003). In breast cancer screening (Hanser, 2005) the nurse has the roles of educator, health promoter, advocate, researcher, consultant, and direct care provider. The preventive services delivered by nurses in the form of health assessment, screening, and counseling can be integrated into comprehensive health promotion and protection activities at the community level, including worksites (Chernecky and End, 2009).

Aim of the study

This study aim is to assess the effect of a nursing intervention program leading to health decisions for breast cancer screening among working women. The specific objectives included:

1. Assessing knowledge, attitude, practices, and health beliefs regarding breast cancer screening;
2. Planning, implementing, and evaluating a structured nursing intervention meeting identified needs and beliefs.

It was hypothesized that the educational nursing intervention program will improve women knowledge, modify their attitude, and empower them to take informed health decisions for breast cancer screening.

2. Subjects and Methods:

Study design

A quasi-experimental design, with pre-post assessment was utilized to conduct the study.

Setting

The study was conducted in three types of work places. These included two pharmaceutical companies, two food processing industries, and a textile factory.

Sample

A convenience sample of working women in the previously mentioned settings at the time of the study was recruited. The only inclusion criterion was being at age 35 years and above. Their total was 520 women.

Tools of data collection

The data collection tools included a self-administered assessment questionnaire, health beliefs assessment rating scale, attitude rating scale, Breast self-examination observation checklist, and mammography card. Face and content validity of the tools were ascertained by a panel of experts in community health nursing, medical-surgical nursing, radiation oncology, and nuclear medicine who revised the tools for clarity, relevance, applicability, comprehensiveness, and ease for implementation. According to their opinion, minor modifications were applied.

Five tools were used for data collection.

Self-administered knowledge assessment questionnaire was developed by the researchers in an arabic language, based on literature review and experts' opinions. It covered woman's personal and job characteristics, menstrual and obstetric history, previous breast disease, family cancer history, previous practice of breast self-examination and mammography. It also included a section of 20 multiple choice questions for assessment of woman's knowledge regarding breast cancer, incidence, symptoms, risk factors, methods of early detection, and methods of prevention.

Health beliefs assessment rating scale modified from Attia et al (1997) by the researchers. It included four items, namely perceived susceptibility, perceived health benefits, barriers to practice, and misconceptions regarding breast self-examination. It consisted of 16 statements on a two-point scale: agree and disagree.

Attitude rating scale to assess woman's attitude towards mammogram screening. It was modified from El-Hadad (1995) by the researchers. The scale consisted of 16 statements on a 3-point Likert scale: agree, uncertain, and disagree.

Breast self-examination observation checklist developed by Long et al (1993) it was used for assessing women's practice of breast self-examination. It involved 10 steps marked as not done, done incorrectly, and done correctly.

Mammography card designed by the researchers for recording woman's attendance to mammography center. It contains the title of the program, name of the woman, name of the workplace, and name of the center.

The knowledge, attitude, health belief, and breast self-examination were used for assessment of the effect of the intervention through pre-post testing.

Scoring

Knowledge: 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 50% or more and unsatisfactory if less than 50%.

Health belief scale: A two-point scale was used, agree and disagree, scored 1 and 0 respectively. 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. More than 60% was considered positive heath belief, and less than 60% was considered negative heath belief.

Attitude scale: Each statement has 3 levels of answers: “agree”, “uncertain”, and “disagree.” These were respectively scored 3, 2, and 1. The scores of the items were summed-up and the total divided by the number of the items, giving a mean score. These scores were converted into a percent score. The attitude was considered positive if 60% or higher, and negative if less.

Observation checklist of breast self examination: A three-point scale were used: not done=0, done incorrectly=1 and done correctly=2. The total score of practice was 20 points. For successful performance of breast self examination, the women must get 20 points.

Pilot study

A pilot study was carried out on 52 working women (10% of the total sample) from the pharmaceutical industries whom were not included later in the study sample. The aim of the pilot study was to test clarity and simplicity of the tools. Necessary modifications were carried out based on the findings of the pilot study and expert's opinion to develop the final form of the tools.

Intervention program

The nursing intervention program was designed by the researchers based on the results obtained from the study tools and findings of similar research. Its aim was to provide accurate knowledge about breast cancer, early detection and screening measures, in addition to acquiring practice skills, and modifying related misconceptions. It was revised and modified to fit cultural and socio-demographic aspects of the study sample. It covered knowledge, beliefs, attitude and practice. This program was reviewed by experts in community health nursing, medical-surgical nursing, radiation oncology, and nuclear medicine to ascertain its content validity. An illustrated Arabic language booklet was constructed as an educational reference during program implementation and self-learning reference afterwards.

Field work

After securing official permissions to carry out the study, the researchers met with the working women in their workplaces. The aim of the study was explained to them and their informed consent was secured before collecting data. The field work was carried out along a period of 11 months starting from March 2008 to February 2009, five days weekly. The assessment phase lasted for three months. The implementation phase of the program and post-test took eight months.

Program implementation was in the form of small group sessions, the program content has been sequenced through 13 sessions (2 session for pre-test, 9 session for program implementation, 3 session for theory and 6 session for practice and 2 session for post test and observation check list). Group consisted of 30 women chosen from different departments according to work conditions. The lists of participants were prepared and provided to the administration office for agreement, and then printed and distributed to all departments. Sessions were conducted in conference rooms in each workplace. Different educational methods and media were used. Post-tests were conducted at the end of the program, and at two-month follow-up.

Ethical considerations

At the initial interview, each potential subject was informed about the nature, purpose, and benefits of the study, and informed that her participation is voluntary. Confidentiality and anonymity of the subjects were also assured through coding of all data. The researcher assured that the data collected and information will be confidential and would be used only to improve their health and for the purpose of the study.

Statistical analysis

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.

Statistical significance was considered at p-value <0.05.

Limitations of the study

The researchers were faced with many logistic problems and spent much effort to convince and promote the objectives of the study. They were faced with refusals from workplaces, and also from working women due to culturally related fears, pessimism, and wrong beliefs. Also, it was difficult to gather all women at the same time for program implementation, and thus the program had to be repeated several times in the same setting.

3. Results

Table 1 show that the mean age of studied women was 43.2±6.3 years. The majority of women were married (93.1%), and more than half of them had secondary education (56.7%). About two thirds (64.2%) were working in pharmaceutical companies, and less than half (47.3%) of them were exposed to chemical substances at workplace; of these, 66.7% reported using personal protective equipment (PPE). Concerning obstetric history, table 2 indicates about half of the married women in the study sample were Para 3-4 (48.2%). Breast feeding was practiced fully by about two thirds of them (66.3%). The majority (81.1%) of women used contraceptive methods. Previous breast problems were experienced by 14.2% of the women, and about one-tenth of them (9.8%) gave a positive family history of breast cancer. The practice of breast self-examination and clinical breast examination was very low, 4.6% and 6.3%, respectively.

As Table 3 illustrates, slightly less than half (48.8%) of the women reported having heard about early detection of breast cancer. The main source of information was radio and TV (55.5%), followed by physicians (21.3%); none of them mentioned nurses. As for the barriers to practice early detection measures, nearly half of them reported lack of knowledge (51.2%) and fear of diagnosis (49.4%).Meanwhile, 3.7% of them mentioned that there were no barriers.

Table 4 points to statistically significant improvements in women's knowledge about breast cancer and early detection methods after implementation of the intervention program. This improvement continued throughout the follow-up phase. The lowest percentage of satisfactory knowledge before the intervention was related to breast cancer risk factors (0.8%). Overall, 5.4% of the women had total satisfactory knowledge at the pre-test. This increased to 99.6% and 98.7% at the post and follow-up tests, respectively.

Statistically significant improvements were revealed in women's health beliefs and attitudes towards early detection of breast cancer. As Table 5 shows, the perceived health benefits of BSE increased from 26.5% at the pre-intervention phase, to 99.8% at the post phase, and 98.5% at the follow-up phase. Similar improvements were noticed regarding perception of susceptibility, barriers, and misconceptions. The same table indicates an improvement in positive attitudes towards early detection of breast cancer, from 10.4% at the pre-intervention phase, to 98.7% at the post phase, and 97.1% at the follow-up phase.

Table 6 indicates that about three-fourth of studied women were observed to successfully perform BSE at the post (73.3%) and follow-up (72.9%) phases of the intervention, compared to none at the pre-test, and the differences were statistically significant (p<0.001). Also, the practice of mammogram increased from 4.2% at the pre-intervention to 17.7% at the follow-up (p<0.001).

Table (1): Personal and job characteristics of women in the study sample (n=520)

	Frequency	%
Age (years):		
35-	183	35.1
40-	149	28.7
45-	82	15.8
50+	106	20.4
Mean±SD	43.2±6.3	
Educational level		
Basic education	37	7.2
Secondary education	295	56.7
Higher education	188	36.1
Marital status:		
Married	484	93.1
Unmarried (single/divorced/widow)	36	6.9
Industry:		
Pharmacy	334	64.2
Food	114	21.9
Textile	72	13.9
Chemical exposure:		
Yes	246	47.3
No	274	52.7
Use of PPE (n=246):		
Yes	164	66.7
No	82	33.3

Concerning the factors related to women's practice of mammography at the follow-up phase, Table 7 points to statistically significant associations with workplace (p=0.009), age (p<0.001), and family history of breast cancer (p<0.001). It is evident that the highest practices were among women working in

pharmaceutical companies, those with age 45 of older, and those with positive family history of breast cancer.

Table (2): Obstetric, medical and family history of women in the study sample (n=520)

	Frequency	%
Parity (n=500):		
0	25	5
1-2	217	43.4
3-4	241	48.2
>4	17	3.4
Breast fed (n = 475):		
Yes all children	315	66.3
Yes some of the children	136	28.6
Never breast fed	24	5.1
Use of contraception (n = 475):		
Yes	385	81.1
No	90	18.9
History of:		
Previous breast problems	74	14.2
Family history of breast cancer:	51	9.8
Practice of breast self examination	24	4.6
Practice of clinical breast examination	33	6.3

4. Discussion:

Breast cancer is the most common type of cancer in women and ranks second only to lung cancer as a cause of cancer related deaths. Recent studies have shown that deaths from breast cancer for women in their forties can be reduced by 17 percent and by at least 30 percent for women ages 50-69, if they follow breast cancer screening recommendations, including routine mammography, regular examinations by a physician, and monthly breast self exams (Hoffman, 2004). Thus, the best way to fight breast cancer is through early detection, and women who find breast cancer lumps early on are far more likely to successfully defeat the disease (Smith, 2006). The present study aim was to assess the impact of a nursing intervention leading to health decisions for breast cancer screening among working women.

Most of the present study women were in the age group 35 to less than 45, which is the age of rise of breast cancer risk. In this regard, Hoskin and Makin (2003) stated that age was by far the most important risk factor for breast cancer, and that the risk increases tenfolds between 30 and 50 years. This doubles again by the age of 70 to 1:300. Also, Largent et al (2005) mentioned that 94% of new cases of breast cancer reported during 1996-2000 occurred in women age 40 and older. The risk of a positive family history has been confirmed previously by Yipch et al, 2008 who mentioned that breast cancer risk was higher among women whose close blood relatives have this disease.

The obstetric data of the present study women indicate that most of cases are at low risk of developing breast cancer. The majority were porous, mostly multiparous, and breastfed their infants, either all or some. Also, the family history of breast cancer was less than 10%. In line with this, Manetta (2004) claimed that the risk of breast cancer increased among women who have had no children. As for breast feeding, the American Cancer Society (2008) reported that it might slightly lower breast cancer risk, especially if breast feeding is continued for 1.5 to 2 years.

On the other hand, the majority of women in the present study reported the use of contraception. This would constitute a risk factor for breast cancer if hormonal methods are used. The association between the use of oral contraceptives and the development of breast cancer has been documented previously (Suzanne et al, 2006).

Table (3): Sources of information about early detection of breast cancer and related barriers as reported by women in the study sample (n=520)

	Frequency	%
Heard about methods of early detection:		
Yes	254	48.8
No	266	51.2
Sources of information (n=254):@		
Radio and TV	141	55.5
Physician	54	21.3
Newspapers	42	16.5
Relative or friends	23	9.1
Nurse	0	0.0
Barriers to practice of early detection measures: @		
Lack of knowledge	266	51.2
Fear of diagnosis	257	49.4
Feel not susceptible	111	21.3
Lack of time	99	19.0
Fatalistic attitude (dependence on Allah)	61	11.7
Cost of diagnostic procedures	61	11.7
Embarrassment	59	11.3
Possibility of errors of doctors and mammogram	32	6.2
No barrier / should do it	19	3.7

(@) Not mutually exclusive

Table (4): Women's had satisfactory knowledge about breast cancer and early detection throughout program phases

Satisfactory Knowledge About:	Program phase						X^2 Test (p-value) Pre-post	X^2 Test (p-value) Pre-FU
	Pre (n=520)		Post (n=520)		FU (n=520)			
	No.	%	No.	%	No.	%		
Breast cancer:								
Definition	200	38.5	520	100.0	517	99.4	462.2 <0.001*	451.26 <0.001*
Incidence	119	22.9	500	96.2	517	99.4	579.31 <0.001*	641.15 <0.001*
Symptoms	64	12.3	508	97.7	513	98.7	765.87 <0.001*	784.82 <0.001*
Risk Factors	4	0.8	517	99.4	489	94.0	1012.19 <0.001*	907.16 <0.001*
Prevention	38	7.3	518	99.6	511	98.3	890.42 <0.001*	863.18 <0.001*
Total	8	1.5	517	99.4	507	97.5	996.56 <0.001*	957.78 <0.001*
Early detection:								
Methods	39	7.5	518	99.6	517	99.4	886.96 <0.001*	883.02 <0.001*
Breast self exam	80	15.4	520	100.0	520	100.0	762.67 <0.001*	762.67 <0.001*
Clinical breast exam	137	26.3	520	100.0	520	100.0	606.27 <0.001*	606.27 <0.001*
Mammogram	79	15.2	520	100.0	520	100.0	765.68 <0.001*	765.68 <0.001*
Total	48	9.2	520	100.0	520	100.0	864.23 <0.001*	864.23 <0.001*
Total knowledge	28	5.4	518	99.6	513	98.7	925.78 <0.001*	906.19 <0.001*

(*) Statistically significant at p<0.05

Table (5): Women's beliefs and attitudes towards early detection of breast cancer throughout program phases

	Program phase						X^2 Test (p-value) Pre-post	X^2 Test (p-value) Pre-FU
	Pre (n=520)		Post (n=520)		FU (n=520)			
	No.	%	No.	%	No.	%		
Positive perception of:								
Susceptibility	244	46.9	519	99.8	511	98.3	372.13 <0.001*	344.56 <0.001*
Health benefits	138	26.5	519	99.8	512	98.5	599.96 <0.001*	573.85 <0.001*
Barriers to practice	67	12.9	519	99.8	513	98.7	798.65 <0.001*	775.38 <0.001*
Misconceptions	137	26.3	520	100.0	520	100.0	606.27 <0.001*	606.27 <0.001*
Total attitude Positive	54	10.4	513	98.7	505	97.1	816.99 <0.001*	786.74 <0.001*

(*) Statistically significant at p<0.05

Table (6): Women's practices of early detection measures of breast cancer throughout program phases

	Frequency	%
Observed adequate practice of breast self exam:		
Pre	0	0.0
Post	381	73.3
Follow-up	379	72.9
X^2 (p-value): pre-post	601.27 (<0.001*)	
X^2 (p-value): pre-FU	596.31 (<0.001*)	
Practice of mammogram:		
Pre	22	4.2
Follow-up	92	17.7
X^2 (p-value): pre-FU	48.27 (<0.001*)	

(*) Statistically significant at p<0.05

Table (7): Relation between women's practice of mammography at follow-up phase and some personal and job characteristics

personal and job characteristics	Mammography				X^2 Test	p-value
	Done (n=92)		Not done (n=428)			
	No.	%	No.	%		
Workplace:						
Pharmaceutical	69	20.7	265	79.3		
Food	19	16.7	95	83.3	9.38	0.009*
Textile	4	5.6	68	94.4		
Age:						
35-	4	2.2	179	97.8		
40-	28	18.8	121	81.2		
45-	31	37.8	51	62.2	59.92	<0.001*
50+	29	27.4	77	72.6		
Marital status:						
Married	83	17.1	401	82.9		
Unmarried	9	25.0	27	75.0	1.42	0.23
Educational level:						
Basic education	3	8.1	34	91.9		
Secondary education	51	17.3	244	82.7	3.19	0.20
Higher education	38	20.2	150	79.8		
Family history of breast cancer:						
Positive	23	45.1	28	54.9		
Negative	69	14.7	400	85.3	29.16	<0.001*

(*) Statistically significant at p<0.05

Added to this risk is the occupational exposure, where about two thirds of the present study women were working in pharmaceutical industries, and about half reported chemical exposure at workplace, with minor use of personal protective equipment. Although these exposures, besides the lack of personal protection, would pose significant risks on working women, still occupational exposures have not been studied thoroughly in relation to breast cancer (Susan et al, 2003).

Slightly less than half of the present study women heard about early detection of breast cancer.

However, only less than 5% of them reported practicing breast self examination (BSE). This deficient practice could be explained by fear of women from diagnosis of breast cancer, lack of knowledge of its significance, or related misconceptions. Other studies reported higher rates of practice. Warner et al (2003) reported that 34% of studied women practice BSE monthly and 16% practiced it anytime. Also, in Jenny and Cielito (2002) study, 38.3% of the sample reported ever performing BSE. The discrepancy between these studies and the present one could be attributed to women higher health awareness, supporting health campaigns, and health insurance for screening measures and early detection in developed countries.

According to the present study, slightly more than half of the women reported that their source of information for breast cancer and early detection was the radio and TV. Physicians were mentioned by about one-fifth of them, while none of them mentioned nurses. The finding is alarming and points to deficiency in health care providers' educational roles. In contradiction with these results, Sief and Aziz (2000) reported that the main source of information among studied women was peers (47.8%) while media as TV, radio, and news papers came second in rank (30.4%). But still the two studies agree on the deficient role of healthcare providers.

As regards the barriers to practice early detection measures, more than half of the present study women reported lack of knowledge and fear of the consequences. This lack of knowledge was quite evident at the pre-test, where only 1.5% of them had satisfactory knowledge about breast cancer, and 9.2% had satisfactory knowledge about early detection. However, there were statistically significant improvements in knowledge at the post and follow-up phases of the intervention. These findings are in congruence with Abdulbari et al (2002) who reported participants' knowledge was mostly low and unsatisfactory. bEl-Hossiny (2002) reported slightly better results regarding definition of cancer breast, its signs and symptoms, and diagnostic methods.

Concerning health beliefs related to early detection, the present study showed that before the program only less than half of the women perceived susceptibility to breast cancer, and only about one-fourth perceived the health benefits of early detection. Altogether perceived susceptibility is a significant variable influencing public awareness and participation in more preventive actions. Women's perception of susceptibility, health benefits, and positive look at barriers and misconceptions that would discourage them from seeking screening and treatment demonstrated statistically significant improvements at the post and follow-up tests. In contradiction with these findings, Attia et al (1997) reported little improvement in students' perceived susceptibility, perceived health benefits and perceived barriers to practice after viewing a BSE educational film. The difference with the present study implies that perceived barriers may be positively modified if suitable learning strategies are chosen.

The present study intervention involved training participating women in the practice of breast self examination. Although a few of them reported practicing it, none had an adequate practice in the pre-intervention phase. Meanwhile, statistically significant improvements were revealed at the post and follow-up phases, with about three-fourth of them having adequate practice. This result is in congruence with Leight et al 2003) who stated that individual training in BSE with guided practice improved both the depth of palpation and the search duration of BSE. On the same line, Jane (2005) reported that an intervention program significantly increased both BSE frequency and accuracy among women in the experimental group.

Concerning actual practice of mammography, only 4.2% of the present study women reported that they had it before the intervention program. This increased to about one-fifth at the follow-up phase, with a statistically significant difference. This finding points to success of the intervention in helping participating women in decision-making regarding their health, and in having a positive impact on their health behavior. However, despite this improvement, still more than four-fifth of the women did not decide to take the test. This could be due to lack of time, or due to the costs of this test. Therefore, Abdulbari et al., 2002) recommended the provision of comfortable, supportive settings for screening that positively alter women's fears and concerns.

As for the characteristics of the women who reported practicing mammography at the follow-up phase, the present study revealed statistically significant relations with workplace, age, and family history of breast cancer. More women working in pharmaceutical industries, with age 45 or older, and with positive family history reported having the test. All these three variables reflect higher risk of breast cancer. Therefore, women having known these risk factors through the intervention program were encouraged to take the test. In agreement with this finding, Murabito et al., 2001) found that women with a family breast cancer history reported higher practice of mammography compared to other women. On the other hand, the present study could not reveal any relation of statistical significance between the

practice of mammography and woman's educational level. This finding is incongruent with Abdel-Fattah (2000) who stated that practice of early detection was positively associated with educational level. The lack of association in the present study could be attributed to the fact that the majority of the sample had secondary or higher level of education.

5. Conclusion and Recommendations

The results of this study demonstrated that working women had deficient knowledge, and negative perceptions related to breast cancer and its early detection. Their practice of breast self-examination and mammography was very low. The nursing intervention program had a positive effect on women's knowledge, practice, health beliefs and attitude towards breast cancer screening and early detection; it empowered about one fifth of women to take informed health decision for having mammography as a screening measure for breast cancer.

In the light of these findings, continuous and comprehensive workplace educational health programs are recommended to provide working women sound information about risk factors, breast cancer screening and early detection methods. Supportive health insurance should be provided for working women to encourage and empower them to practicing screening procedures. Training programs should be provided to nurses in order to have an active role in empowering women to take informed health decision related to breast cancer screening and early detection. Further research studies with broader range of occupational settings are suggested.

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In Vitro Maturation of Camel Oocytes As Affected By Different Media during Breeding and Non-Breeding Seasons

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Abstract: A total number of 220 clinically healthy she-camel was used in this study. The age of these camels varied from 5 to 10 years and their weights were approximately 500-600 kg. Two experiments were carried out. The first experiment aimed to define the effect of different seasons of the year on follicular fluid components and ovarian activity either in the right or left ovary. The second experiment designed to define the effects of various maturation media (TCM 199, Ham's F-10, Basal and Hank's) on the *in vitro* maturation of camel oocytes during breeding and non-breeding seasons. In the first experiment, the obtained results showed that ovary weight and number of corpora lutea were significantly ($P < 0.05$) higher during spring, winter and autumn seasons, than summer season. Numbers of the normal follicles were significantly ($P < 0.05$) higher during spring, while the atretic follicls were significantly ($P < 0.05$) higher during summer season than other seasons. Oocytes recovery, compact oocytes complexes (COC's) and partially denuded cumuls oocytes (PDCO) were significantly ($P < 0.05$) higher during autumn, while expanded cumulus oocytes (ECO) and denuded cumulus oocytes (DCO) were significantly ($P < 0.05$) higher during spring and winter seasons than other seasons of the year. The highest ($P < 0.05$) activities of follicular fluid aspartate – aminotransaminase (AST), alanine – aminotransaminase (ALT), alkaline phosphatase (ALP) and acid phosphatase (ACP) enzymes were recorded during summer and the lowest ($P < 0.05$) activity was recorded during spring season. The highest ($P < 0.05$) values of follicular fluid potassium and calcium were recorded during winter and the lowest ($P < 0.05$) values were recorded during summer season. Testosterone concentration was significantly ($P<0.05$) higher, however cholesterol concentration was significantly ($P < 0.05$) lower during summer season, meanwhile oestradiol-17β concentration was significantly ($P < 0.05$) higher during winter season than other seasons of the year. Ovary weight, number of the corpora lutea (CL) and number of the normal follicles in the left were significantly ($P< 0.05$) higher than the right ovary, while the number of the atretic follicles in the right was significantly ($P<0.05$) higher than the left ovary. Oocyte recovery and oocyte status (COC's, PDCO, ECO and DCO) in the left ovary were significantly ($P < 0.05$) higher than the right one. In respect to ovary side, AST, ALT, ALP, ACP, sodium and testosterone concentration of follicular fluid in the left ovary were significantly ($P < 0.05$) lower than the right one. Cholesterol, potassium, calcium, inorganic phosphorus and oestradiol-17β concentrations in the left were significantly ($P < 0.05$) higher than the right ovary. In the second experiment, results revealed significantly ($P<0.05$) higher cumulus expansion, meiosis metaphase I (MI) and metaphase II (MII) than the non-breeding season . When the type of culture media there was no differences in cumulus expansion except with basal medium which produce the lowest incidence in both breeding and non-breeding season. In breeding season, TCM-199 medium showed the highest rate ($P<0.05$) of MII oocytes, while in non-breeding season, TCM-199 and Ham's F-10 media showed the highest rates ($P<0.05$) of MII oocytes.

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Key words: Camels, season, ovary, follicular fluids, oocytes, *in vitro* maturation.

1. Introduction

Camels are induced ovulations and exhibit follicular cycles with follicles developing and regressing successively and ovulation will occur only when mating takes place (Elias *et al.*, 1984 and Ismail, 1987). Both the dromedary and bactrian camels are regarded as seasonal breeders, with a relatively short breeding season, based on the seasonal, distribution of births and the status of ovarian activity (Shalash, 1980). Outside the breeding season, mating activity ceases and the ovaries are inactive or only have a few small follicles. However, there are conflicting reports about the beginning and length of the seasonal activity in the dromedary, increased breeding activity has been reported to occur in March and August in Sudan (Musa and Abusineina, 1978), December to March in Pakistan

(Yasin and Wahid, 1957), December to April in Egypt (Shalash, 1987) and from November to April in most of Arabian countries (Tibary and Anouassi, 1996). This is generally during the period of low climatic temperature, rain and better grazing conditions.

Application of assisted reproductive technologies such as artificial insemination, embryo transfer and *in vitro* production of embryos, which as in most domestic species could offer an apportunity to better understanding of factor that regulate reproduction in camels. In this respect, the application of the *in vitro* embryo production technology can facilitate the study of basic mechanisms regulating reproduction in camels.

Ovaries from slaughter house being the cheapest and most abundant source of oocytes are used for large scale production of mature oocytes in most of the animal species. As such, extensive studies on *in vitro* oocyte maturation of many domestic species have lead to improved culture conditions, so that a large percentage of oocytes successfully complete nuclear maturation (Eppig, 1991).

The *in vitro* maturation technique (*IVM*) needs a large number of good quality oocytes, which mainly depend upon the available number of follicles on the ovary in addition to the method of recovery. The regulation of oocyte maturation not only affected the proportion of oocytes capable of undergoing maturation, but also their subsequent fertilization and development (Bavister *et al.,* 1992). Few authors have studied *in vitro* maturation and fertilization of camel oocytes.

Therefore, the present study included two experiments. The first experiment, aimed to investigate the effect of different seasons of the year on ovarian activity of the dromedary she-camel. The second experiment, intended to define the effects of various maturation media during breeding and non-breeding seasons on the *in vitro* maturation of she-camel oocytes.

2. Materials and Methods

The present study was conducted in the Laboratory of Physiology, Department of Animal Production, Faculty of Agriculture, Mansoura University, in co-operation with Animal Production Research Institute, Dokki, Giza, Egypt.

The experimental work was carried out in the Private Camel's Farm, Belbies City, Sharkiya Governorate, located in the North Eastern part of the Nile Delta (30 ˚N). A total number of 220 clinically healthy she-camel was used in this study. The age of these camels varied from 5 to 10 years and their weights were approximately 500-600 kg. The present work included two experiments. The first experiment, aimed to investigate the effect of different seasons of the year on follicular fluid components (AST, ALT, ALP, ACP, cholesterol, sodium, potassium, calcium, inorganic phosphorus, testosterone and oestradiol-17ß hormone) and ovarian activity (ovary weight, number of corpora lutea, number of oocytes and oocyte status) either in the right or left ovary of the dromedary camel. The second experiment designed to define the effects of various *in vitro* maturation media (TCM 199, Ham's F-10, Basal medium and Hank's) on the maturation rate of she-camel oocytes during the breeding and non-breeding seasons.

Minimum and maximum values of air temperature (˚C), relative humidity (%). temperature-humidity index (THI) and length of daylight (hours) of the different seasons of the year are shown in Table 1. The temperature - humidity index (THI) was estimated according to Livestock and Poultry Heat Stress Indices (LPHSI, 1990), using the following formulae:

THI=db ˚F – (0.55-0.55 RH) (db°F-58.00) where db ˚F=dry bulb temperature in Fahrenheit and RH=relative humidity (RH% / 100). The obtained values of THI were classified as follows: less than 72=absence of heat stress, 72 to < 74 moderate heat stress, 74 to < 78=severe heat stress and over 78 very severe heat stress.

First experiment

1. Ovarian activity:

1.1. Ovaries collection:

A total number of 440 ovaries collected from 220 clinically healthy she-camels was used in this study. Two ovaries (right and left) from each camel were collected immediately after slaughtering within 30-60 minutes and washed by sterile warm normal saline (0.9% NaCl) containing 100 IU penicillin G-sodium and 100 µg streptomycin sulfate /ml. The ovaries were kept in pairs in plastic bags containing saline, then transported to the laboratory in a thermos containing sterile normal saline at 30 - 35˚C. Weight and number of corpora lutea, follicles, oocytes and oocytes status per ovary, were recorded. The ovaries were excised and submerged in *in vitro* fertilization *(1VF)* dishes with saline solution (0.9% NaCl). Number of oocytes was recorded from the normal visible follicles (5-10 mm) on ovaries existed from camel then punctured and the content was expelled into a disposable Petri dish (30X60mm). The follicular fluid was centrifuged at 600 *g* for 15 minutes to remove the cellular debris and the supernatant fluid was pooled in ach class separately stored at - 20 °C to fulfill all required biochemical analysis.

Table (1): Mean air temperature (°C), relative humidity (%), temperature-humidity index (THI) values and daylight length, during the different seasons of the year.

Seasons	Air temperature (°C)		Relative humidity (%)		Temperature-humidity index (THI)		Length of daylight (hours)
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	
Winter	8.86±0.21	19.15±0.35	48.62±0.35	64.33±1.15	45.11	64.81	11.55
Spring	13.60±0.18	24.16±0.18	37.41±0.43	52.64±1.21	55.96	70.93	14.13
Summer	20.84±0.32	34.30±0.46	38.83±0.48	53.66±0.95	65.64	84.63	15.24
Autumn	15.43±0.12	28.62±0.42	42.67±0.62	58.42±1.32	59.21	77.68	13.00

1.2. Ovarian weight (g):

After removal of the extraneous tissues, each ovary (right or left) was weighed using an electric balance.

1.3. Number of follicles and corpora lutea:

Immediately after slaughter, ovaries were removed on and all the normal visible follicles (5-10 mm) or corpora lutea either left or right ovaries were counted.

1.4. Follicle type:

The follicle was differentiated according to the nature of the contained follicular fluid as follow:

a. Normal follicles: were target, transport, almost spherical, easily squeezable and thick wall.
b. Atretic follicles: were opaque, nearly spherical and relatively thin walled.

2. Follicular fluid components:

AST, ALT enzyme activities were determined colourimetrically using the method described by Reitman and Frankle (1957), while ALP and ACP activities and cholesterol concentration were determined colourimetrically using commercial kits purchased from Bio-Merieux (Marcy L'Eltoile, Charbonnieres, Les Bains, France) according to Graham and Pace (1967). Inorganic phosphorus, sodium, potassium and calcium concentrations were determined according to the method described by Kuttner and Liechtenstein (1930), Trinder (1951), Sunderman Jr. Sunderman (1958) and Gindler (1972), respectively. Testosterone and oestradiol-17ß hormones were determined by Radiommunoassay Technique (RIA) using commercial kits (Diagnostic Products Corporation, Los Angles, USA).

3. Oocytes collection:

Oocytes were collected using aspiration from the antral follicles (5–10 mm in diameter) either left or right ovary individually using 5 ml syringe and 20 gauge needle. Before commencing aspiration, the needle and syringe are first primed with approximately 0.25 ml of aspiration medium. After aspiration, the contents of the syringe were slowly dispelled into sterile Petri dishes (30x60mm) with minimum disruption of the cumulus oocytes complex. Repeated aspirations of follicles were performed to collect oocytes into the syringe. Number of oocytes recovered from follicles into each of right or left ovaries was recorded using Stereo-microscope.

3. l. Oocytes recovery:

Oocytes yield from aspiration of the follicles in each of right or left ovaries was recorded. The recovery rate was determined as the percentage of oocytes in proportion to each of the total vesicular follicles according to Mayer *et al.* (1986) as the following formulea:

$$\text{Recovery rate} = \frac{\text{No. of oocytes recovered}}{\text{No. of vesicular follicles}} \times 100$$

3.2. Oocytes evaluation:

The oocytes were evaluated in respect to both investment and ooplasm granulation as the method described by Madison *et al.* (1992).

3.2.1. Cumulus evaluation

a. Compact cumulus oocytes complexes (COC's) :
Oocytes with complete compact dense cumulus oophrus more than 3 layers (grade I).
b. Partially denuded cumulus oocytes (PDCO) :
Oocytes with compact cumulus layer not completely surrounding the oocyte or less than 3 layers (grade II).
c. Expanded cumulus oocytes (ECO) :
Oocytes surrounded by expanded layers of cumulus cells appearing as scattered clamps in the matrix (grade III).
d. Denuded cumulus oocytes (DCO):
Oocytes enclosed only by the zona pellucida without cellular investment (grade IV).

3.2.2. Ooplasm evaluation:

a. Even ooplasm:

Granulation of ooplasm given the oocytes a dusty appearance and the ooplasm evenly fill the zona pellucida.

b. Uneven ooplasm:

Granules clumped or uneven distributed in ooplasm and the ooplasm remarkably fills the zona pellucida.

c. Shrunken ooplasm:

Ooplasm shrunken away from the zona pellucida or not evenly filling the zona. Ooplasm also looks degenerated with fragment empty zona pellucida.

The oocytes of category I and II and evenly granulated dark ooplasm were selected to undergo *in vitro* maturation (usable oocytes), but category III and IV were discarded (unusable oocyte).

Second experiment

In vitro maturation of camel oocytes:

1. Media:

Four types of maturation media (TCM-199, Ham's F-I0, Hank's and Basal media) were used for oocytes washing and maturation obtained in a liquid form (from Egyptian Organization for Biological Product and Vaccine, Agoza) and stored in the refrigerator at 5°C till usage.

Preparation of media:

All media (TCM-199, Ham's F-I0, Hank's and Basal media) supplemented with 10 mg L.glutamine, 100 IU Penicillin G-Sodium and 50 µg Streptomycine100ml /ml were used. Value of pH was measured by pH meter and adjusted to pH 7.4 using NaOH (Sigma, Chemical P.O Box 14508 ST. Louis, MO 63178, USA). Each medium was sterilized using 0.2 µm millipore filter and equilibrated in CO_2 incubator (5% CO_2) with relative humidity of 38.5 - 39°C for at least 2 hours prior to use.

2. Cultivation of oocytes:

The selected oocytes were collected from the left ovary (according to the results of the first experiment) and washed three times in all the maturation media using line polished Pasteur pipette before being injected finally in four wells culture dishes, each containing 500-750 µl of the culture media.

3. Assessment of maturation:

The judgment of oocytes maturation was based on cumulus expansion and nuclear maturation.

3.1. Cumulus expansion:

The cumulus expansion was determined after oocytes incubation under Stereo microscope. The criteria of assessing the cumulus expansion was done according to Chauhan *et al.* (l999) as follow:

a. Expanded cumulus : cell mass was expanded away from the zona pellucida.
b. Non expanded cumulus : cell mass was tightly adherent to the zona pellucida.

3.2. The nuclear maturation:

For the judging of the nuclear maturation, cumulus compact oocytes complexes (COC's) were transferred to small plastic tube containing 3% sodium citrate solution followed by repeated agitation for the denudation of the oocytes (Carolan *et al.*, 1994). The contents of the tube were transferred to a new 35 mm Petri dish and the demanded oocytes were mounted on a glass slide with a cover slip supported by droplets of paraffin Vaseline mixture. Thereafter, oocytes were fixed and cleared with ethanol acetic acid 3:1 at 4 ˚C for 24 h , and stained with aceto-orcein (1% orcein in 40% acetic acid) for 25 minutes. After that oocytes were rewashed with a fresh fixative and examined under light microscope (Ganguli *et al.*, 1998). The stage of nuclear maturation was described as follows:

a. Immature: Germinal vesicle stage with intact nucleus.
b. Intermediate: Paired or bivalent chromosomes were observed within nucleus of the oocyte (Metaphase I).
c. Mature: Two groups of unequally spread chromosomes were observed and the polar body set was clustered together (Metaphase II). Thereafter, the oocytes were categorized as post maturation cumulus expansion (Plate 1), germinal vesicle (GV, Plate 2), metaphase I (M1, Plate 3) and metaphase II (MII, Plate 4).

Data were statistically analyzed using least squares Analysis of Variance according to Snedecor and Cochran (1982). Percentage values were transformed to arc-sin values before being statistically analyzed. Duncan's multiple range test (Duncan, 1955) were used for the multiple comparisons.

3. Results and Discussion:

First experiment

1. Temperature-humidity index (THI):

The temperature-humidity index (THI) estimated in Table 1 indicated exposure of she-camel to very severe heat-stress during summer (non-breeding season), severe heat stress during autumn and absence of the heat-stress during winter and spring seasons (breeding season).

2. Ovarian activity:

2.1. Ovary weight and CL number:

Data presented in Table 2 showed that the effect of season of the year on ovary weight was significant ($P < 0.05$). The highest ($P < 0.05$) value of ovary weight was recorded during autumn and winter and the lowest ($P < 0.05$) values was recorded during summer and spring seasons. Similar trends were recorded by Abdoon and Omima (2006) and Sarhan (2007).

With regard to ovary side, the left and right ovary weight in the dromedary she-camel was significant ($P < 0.05$). In the left ovary, the highest ($P < 0.05$) weight was obtained during autumn followed by those of winter and the lowest ($P < 0.05$) weight during summer. While, in the right ovary, the highest and lowest weights were recorded during winter and summer, respectively. These results are in agreement with those of Yahaya *et al.* (1999) who showed that the right ovary was heavier ($P<0.01$) and had more follicular fluid ($P<0.01$) than left ovaries. In general, ovary weight, increases with ovarian activity (Djang *et al.*, 1988 and El-Wishy, 1992). Ovary side showed significantly effects on the ovary weight although ovary weight was significantly ($P < 0.05$) higher in the left than in the right ovary during all seasons of the year.

The mean number of CL per ovary of dromedary she-camel was significantly ($P < 0.05$) between seasons. The highest ($P < 0.05$) number of CL was obtained during autumn followed by that of spring and the lowest ($P < 0.05$) during summer and winter seasons. The present results are in agreement with those of Yahaya *et al.* (1999) and Sarhan (2007) who showed that, the number of corpora lutea increased during November and December (breeding season). Ovaries examined had 2 active corpora lutea and no ovaries were found with 3 corpora lutea. Abdoon (2001) showed that ovaries without a CL possessed significantly ($P < 0.01$) more ovarian follicles and more ($P < 0.05$) small and large follicles.

Ovary side had significantly ($P < 0.05$) effects on the ovary number of CL, being higher in the left than in the right ovary during all seasons of the year.

In general, it is interested to notice that, superiority of the oocytes recovery in both left and right ovaries of the dromedary she-camels was recorded in the breeding season (winter, autumn and spring) as compared to the non-breeding (summer) season. These probably may be due to that the gonadotropic hormonal balance was in favor of the follicular growth stimulation oocyte status in the breeding season but is not in favor of ovulation process.

2.2. Follicles number:

Data presented in Table 2 showed the effect of the number of the normal follicles was significantly ($P< 0.05$) increased during spring as compared to other seasons. While, the number of the atretic follicles was significantly ($P < 0.05$) increased during summer as compared to other seasons. In respect to ovary side, the total number of the normal follicles in the left ovary was significantly ($P < 0.05$) higher and significantly ($P<0.05$) lower the atretic follicles than the right one. Meanwhile, the total number of follicles was significantly ($P< 0.05$) higher during summer and winter than spring and autumn seasons. Similar trends were reported by Amer (2004) and Sarhan (2007).

The number of follicles of the dromedary she-camel on the left and right ovaries was significant ($P<0.05$). The highest number ($P<0.05$) of the normal follicles was obtained during spring and the lowest ($P<0.05$) during summer in the right ovary . While, in the left ovary, the highest ($P<0.05$) number of the normal follicles was recorded during winter and the lowest ($P<0.05$) during summer. Concerning the atretic follicles, the highest ($P<0.05$) number of the atretic follicles was obtained during summer and the lowest ($P<0.05$) during spring in the right or left ovary. These results are in agreement with those of Abdoon (2001), Abdoon and Omamia (2006) and Sarhan (2007) who showed that, the number of small, medium, large and the total number of ovarian follicles were higher ($P < 0.01$) during the breeding than non-breeding season. Similar trends were reported by Amer (2004) and Zeidan *et al.* (2008).

2.3. Oocytes recovery:

Table 3 showed that the mean number of oocytes per ovary collected was significantly ($P<0.01$) lower during summer than the other seasons. Abdoon (2001) showed that, the total recovery of oocytes with compact cumulus was greater ($P < 0.01$) during the breeding season. Similar trends were reported by Amer (2004) and Zeidan *et al.* (2008).

In respect to ovary side, the recovery rate of oocytes was significantly ($P < 0.05$) higher in the left than the right ovary during all the different seasons of the year. Similar trends were reported by Amer (2004) and Zeidan *et al.* (2008).

2.4. Oocytes status:

Data presented in Table 3 showed that the means cumulus oocytes complexes (COC's) and partially denuded cumulus oocytes (PDCO) of the dromedary she-camels increased significantly ($P < 0.05$) during spring, autumn and winter as compared with the summer season. The maximal ($P < 0.05$) numbers of COC's and PDCO was recorded in autumn season and the minimal ($P< 0.01$) numbers was recorded in summer one. The expanded cumulus oocytes (ECO) and denuded cumulus oocytes (DCO) were significantly ($P < 0.05$) higher during spring and

winter than the summer and autumn seasons. The maximal ($P < 0.05$) numbers of the ECO was recorded during spring and the minimal ($P< 0.05$) numbers was recorded in autumn season. Meanwhile, the maximal ($P < 0.05$) numbers of the DCO was recorded in winter and the minimal ($P<0.05$) numbers was recorded in summer. Similarly, Amer (2004) and Sarhan (2007) found that the highest numbers of COC's and PDCO was recorded during autumn and winter and the lowest numbers was recorded during summer and spring seasons. While, the highest ECO and DCO were recorded during spring and winter and the lowest during summer and autumn seasons.

Regarding side of ovary, the numbers of COC's , ECO, PDCO and DCO oocytes of the dromedary she-camels were significantly ($P < 0.05$) higher in the left than the right ovary during the different seasons of the year. Sarhan (2007) showed that the ovary side had significant ($P < 0.01$) effect on the oocytes status being higher in the breeding than the non-breeding season. Similar trends were reported by Amer (2004) and Zeidan *et al.* (2008).

Table (2): Means of the right and left ovaries weight, number of corpora lutea and number of the normal and atretic follicles of the dromedary she-camel as affected by different seasons of the year.

Item	Seasons of the year											
	Spring		Mean	Summer		Mean	Autumn		Mean	Winter		Mean
	LO	RO		LO	RO		LO	RO		LO	RO	
Ovary weight (g)	5.07^{a} ± 0.84	4.48^{b} ± 0.76	4.78^{B}	5.02^{a} ± 0.74	4.46^{b} ± 0.71	4.74^{B}	5.51^{a} ± 0.72	4.84^{b} ± 0.58	5.18^{A}	5.49^{a} ± 0.71	5.06^{b} ± 0.65	5.28^{A}
No. of corpora lutea/ovary	2.92^{a} ± 0.25	2.54^{b} ± 0.15	2.73^{A}	2.25a ± 0.65	1.63b ± 0.38	1.94^{B}	3.00^{a} ± 0.74	2.57^{b} ± 0.37	2.79^{A}	2.23^{a} ± 0.34	1.85^{b} ± 0.42	2.04^{B}
Total	5.28^{A}			3.88^{C}			5.57^{A}			4.08^{B}		
No. of the normal ovarian follicles	14.09^{a} ± 1.88	10.64^{b} ± 1.62	12.37^{A}	6.37^{a} ± 0.26	4.25^{b} ± 0.93	5.31^{D}	12.43^{a} ± 1.13	7.28^{b} ± 1.51	9.86^{C}	16.08^{a} ± 1.05	7.07^{b} ± 1.57	11.58^{B}
Total	24.73^{A}			10.62^{C}			19.71^{B}			23.15^{A}		
No. of the atretic ovarian follicles	1.72^{b} ± 0.28	2.86^{a} ± 0.34	2.29^{D}	13.36^{b} ± 1.47	16.82^{a} ± 0.84	15.09^{A}	4.87^{b} ± 0.16	5.37^{a} ± 0.83	5.12^{C}	8.07^{b} ± 0.51	8.85^{a} ± 0.74	8.46^{B}
Total	4.58^{D}			30.18^{A}			10.24^{C}			16.92^{B}		
Total number of follicles	29.31^{C}			40.80^{A}			29.95^{C}			40.07^{A}		

Means bearing different letters within the same row, differ significantly ($P< 0.05$). RO : Right ovary. LO: Left ovary

Table (3) : Means of recovery rate and oocytes status in the right and left ovaries of the dromedary she-camel as affected by different seasons of the year.

Item	Seasons of the year											
	Spring		Mean	Summer		Mean	Autumn		Mean	Winter		Mean
	LO	RO		LO	RO		LO	RO		LO	RO	
No. of ovaries	11	11	11	13	13	13	7	7	7	8	8	8
Oocytes recovery	4.00^{a}	1.44^{b}	2.72^{B}	1.62^{a}	0.33^{b}	0.98^{D}	9.99^{a}	5.14^{b}	7.57^{A}	4.13^{a}	3.50^{b}	3.82^{C}
Investments :												
Compact cumulus oocytes complexes	1.00^{a} ± 0.27	0.18^{b} ± 0.13	0.59^{C}	0.23^{a} ± 0.09	0.10^{b} ± 0.00	0.17^{D}	8.71^{a} ± 0.34	3.72^{b} ± 0.85	6.22^{A}	1.63^{a} ± 0.41	1.26^{b} ± 0.47	1.45^{B}
Partially denuded cumulus oocytes	0.90^{a} ± 0.09	0.09^{b} ± 0.09	0.50^{C}	0.23^{a} ± 0.12	0.00^{b} ± 0.00	0.12^{D}	1.28^{a} ± 0.03	1.00^{b} ± 0.05	1.14^{A}	0.87^{a} ± 0.03	0.55^{b} ± 0.02	0.71^{B}
Expanded cumulus oocytes	1.55^{a} ± 0.47	0.81^{b} ± 0.23	1.18^{A}	0.46^{a} ± 0.18	0.23^{b} ± 0.12	0.35^{C}	0.28^{a} ± 0.18	0.00^{b} ± 0.00	0.14^{D}	0.87^{a} ± 0.41	0.50^{b} ± 0.27	0.69^{B}
Denuded cumulus oocytes	0.55^{a} ± 0.21	0.36^{b} ± 0.21	0.46^{B}	0.70^{a} ± 0.07	0.00^{b} ± 0.00	0.35^{C}	0.14^{a} ± 0.14	0.00^{b} ± 0.00	0.07^{D}	1.13^{a} ± 0.45	0.62^{b} ± 0.18	0.88^{A}

Means bearing different letters within the same row, differ significantly ($P< 0.05$). RO : Right ovary. LO : Left ovary

3. Follicular fluid components:

3.1. Enzymatic activities:

The effects of season of the year and ovary side on the follicular fluid (FF) components of the dromedary camels are presented in Table 4.

The effect of season of the year on AST ALT, ALP and ACP enzymes activity in FF of the camels was significantly (P<0.05) higher during summer than autumn, winter and spring seasons. The highest (P<0.05) value AST, ALT and ALP enzymes was recorded in summer and the lowest (P<0.05) values of was recorded in spring season. While, the ACP enzyme showed higher activity during summer than autumn, spring and winter, since lowest value in autumn season. These results are in agreement with those of Mabrouk *et al.* (1991) and Amer (2004).

Regarding the ovary side, the right ovary showed significantly (P < 0.05) higher activity of AST, ALT, ALP and ACP enzymes than the left one. The active granulosa cells shared to some extent in adding both AST and ALT enzymes to the folliculer fluid of she-camel (Mabrouk *et al.,* 1991). The activity of AST and ALT enzymes in the right ovary might be due to more atrophied and degenerated granulosa cells and the enlargement of cystic size in atretic follicles and to the presence of more active membrane granulosa cells in normal follicles (Abdel Ghaffar *et al.,* 1995). Amer (2004) and Zeidan *et al.* (2008) confirmed that the AST, ALT, ACP and ALP activities in the right ovary were significantly higher than the left one. In contrast ALT, AST, ALP and ACP enzyme activities in the normal follicular fluid of women was higher than in cystic follicles (Causing *et al.*, 1972).

In general, our results showed that the follicular fluid contains high levels of transaminases and phosphatases enzymes which increase with follicular development. So, it is suggested that transaminases and phosphatases enzymes may affect ovarian steriodogenesis. The role of granulose cells in the contribution of these enzymes may be accepted. Likewise, the significant increase in ALP enzyme activity in the follicular fluid might be an indication of atrophy due to lysosomal enzymes that affected the phosphorylated receptors which would lead to atresia (Wise, 1987 and Abdel-Ghaffar *et al.,* 1995).

3.2. Cholesterol concentration:

The effect of season of the year had significantly (P < 0.05) effect on cholesterol concentration in FF of the dromedary she-camels. The highest (P < 0.05) value of follicular fluid cholesterol was recorded during autumn and the lowest (P < 0.05) value was recorded during summer season. Similar trend was reported by Amer (2004), Sarhan (2007) and Zeidan *et al.* (2008) in the dromedary camels. Total cholesterol concentration depends on the environmental and seasonal variations (Sinha *et al.,* 1981). The seasonal variations in cholesterol concentration may be due to the type of feed offered during different seasons of the year. During breeding season (winter), the green fodder was barseem since barseem is a rich source of steroids (Salem, 1980). In addition, decrease of cholesterol during non-breeding season (summer) may be due to lower thyroid activity during rise of environmental temperature which influences cholesterol level.

With regard to ovary side, cholesterol concentration in the right ovary was significantly (P < 0.05) lower than the left one during the different seasons of the year. Similar trends were reported by Amer (2004) and Zeidan *et al.* (2008).

3.3. Minerals concentration:

The effects of different seasons of the year on calcium in FF of the dromedary she-camels was significantly (P<0.05) higher during autumn and winter than spring and summer seasons. The highest (P<0.05) value of follicular fluid calcium concentrations was recorded during autumn and the lowest (P<0.05) value during summer. The increase of calcium concentration at winter may be due to the high calcium values of barseem during breeding season (Ayoub *et al.,* 1972). Similar trends were reported by Amer (2004) and Zeidan *et al. (*2008). These results might be attributed to the atrophy and degenerative changes in the granulose cells during the non-breeding season than the breeding one. This clearly shows to what extent the higher stressful temperature in summer during the non-breeding season exerted unfavorable effects on ovarian activity in terms of lower stimulatory follicular growth than the breeding one.

The effect of season of the year was significantly (P<0.05) higher on sodium concentration in FF during summer than autumn, winter and spring seasons. The highest (P<0.01) value of sodium in FF was recorded during summer and the lowest (P<0.01) value was found during spring season. Similar trend was reported by Amin (1993) and Sarhan (2007). Amer (2004) found also that, sodium concentration of the dromedary she-camels was significantly higher during summer than spring, autumn and winter seasons. The increase of sodium concentration during the heat of summer which indicates a very effective mobilization of the intracellular fluids into extracelular spaces (Rathore, 1986). In addition, these results my be attributed to the combined effect of sodium and chloride absorption from the alimentary tract and kidney, respectively, under the effect of aldosterone hormone which had higher level in summer and this

was accompanied by an increase of plasma sodium level (Yagil and Etzion, 1979).

The effects of different seasons of the year on FF potassium and inorganic phosphorus concentrations were significantly (P<0.05) lower during summer than autumn, winter and spring seasons. The highest (P<0.05) value of FF potassium and inorganic phosphorus concentrations was recorded during spring and winter and the lowest (P<0.05) value was recorded during summer season. Similar trend was reported by Amer (2004) and Zeidan *et al.* (2008) in the dromedary camels.

With regard to ovary side, follicular fluid sodium concentration was significantly (P<0.01) higher, while calcium, potassium and inorganic phosphorus concentrations were significantly (P < 0.05) lower in the right than the left ovary with the different seasons of the year. Similar results were recorded by Amer (2004) and Sarhan (2007) in the dromedary camels. These results may indicate early follicular degeneration of the atretic follicles at the right ovary than in the normal follicles of the left ovary.

Table (4) : Means of the follicular fluid components in the left and right ovaries of the dromedary she-camels during different seasons of the year.

Item	Seasons of the year											
	Spring		Mean	Summer		Mean	Autumn		Mean	Winter		Mean
	LO	RO		LO	RO		LO	RO		LO	RO	
Aspartate-aminotransferase (U/l)	39.41^{b} ± 2.27	68.01^{a} ± 1.61	53.71^{C}	112.38^{b} ± 6.21	129.05^{a} ± 8.59	120.72^{A}	76.18^{b} ± 3.48	96.71^{a} ± 3.43	86.45^{B}	74.74^{b} ± 3.67	95.37^{a} ± 4.92	85.06^{B}
Alanine-aminotransferase (U/l)	6.74^{b} ± 2.15	37.33^{a} ± 2.74	22.04^{C}	38.86^{b} ± 1.68	52.40^{a} ± 1.00	45.63^{A}	33.13^{b} ± 1.19	38.37^{a} ± 0.23	35.75^{B}	35.75^{b} ± 1.51	39.50^{a} ± 1.67	37.11^{B}
Alkaline phosphatase (U/l)	227.21^{b} ± 20.82	342.75^{a} ± 22.04	284.98^{C}	321.57^{b} ± 19.01	369.12^{a} ± 15.23	345.35^{A}	310.54^{b} ± 08.12	324.76^{a} ± 2.26	317.65^{B}	274.83^{b} ± 0.52	300.74^{a} ± 64.14	287.79^{C}
Acid phosphatase (U/l)	2.16^{b} ± 0.19	5.01^{a} ± 0.46	3.59^{B}	2.70^{b} ± 0.58	6.24^{a} ± 0.66	4.47^{A}	2.12^{b} ± 0.03	2.50^{a} ± 0.03	2.31^{C}	2.80^{b} ± 0.15	4.01^{a} ± 0.79	3.41^{B}
Cholesterol (mg/dl)	136.33^{a} ± 12.46	113.33^{b} ± 2.61	124.83^{A}	121.33^{a} ± 09.39	98.66^{b} ± 10.93	110.00^{B}	135.66^{a} ± 10.25	125.66^{b} ± 5.18	130.66^{A}	131.00^{a} ± 2.56	126.00^{b} ± 4.44	128.50^{A}
Calcium (mg/dl)	7.63^{a} ± 1.61	6.10^{b} ± 1.98	6.87^{C}	7.51^{a} ± 0.16	6.46^{b} ± 0.91	6.99^{C}	9.60^{a} ± 0.55	9.00^{b} ± 0.17	9.30^{A}	9.46^{a} ± 0.93	8.43^{b} ± 1.94	8.95^{B}
Sodium (mg/dl)	78.33^{b} ± 13.39	92.33^{a} ± 5.37	85.33^{C}	133.00^{b} ± 4.05	139.50^{a} ± 1.16	136.25^{A}	112.30^{b} ± 2.03	135.33^{a} ± 2.91	123.82^{B}	84.00^{b} ± 8.55	89.00^{a} ± 3.22	86.50^{C}
Potassium (mg/dl)	7.33^{a} ± 0.53	6.60^{b} ± 1.12	6.97^{A}	3.20^{a} ± 0.81	4.10^{b} ± 0.17	3.65^{C}	5.56^{a} ± 0.34	4.40^{b} ± 0.31	4.98^{B}	8.30^{a} ± 0.09	5.93^{b} ± 0.78	7.12^{A}
Inorganic phosphorus (mg/dl)	8.26^{a} ± 0.21	6.63^{b} ± 0.27	7.45^{A}	6.53^{a} ± 0.62	4.93^{b} ± 0.08	5.73^{C}	6.83^{a} ± 0.07	6.36^{b} ± 0.07	6.60^{B}	6.86^{a} ± 0.08	6.53^{b} ± 0.24	6.70^{B}
Testosterone (pg/ml)	11.49^{b} ± 1.18	16.51^{a} ± 1.03	14.00^{D}	19.89^{b} ± 0.25	82.99^{a} ± 2.74	51.44^{A}	15.32^{b} ± 1.29	20.78^{a} ± 0.79	18.05^{C}	12.58^{b} ± 0.17	27.59^{a} ± 3.23	20.09^{B}
Oestradiol 17-β (pg/ml)	138.46^{a} ± 22.18	122.56^{b} ± 41.25	130.51^{A}	138.46^{a} ± 41.25	105.27^{b} ± 19.34	121.87^{B}	136.51^{a} ± 28.12	112.38^{b} ± 25.16	124.45^{B}	142.84^{a} ± 36.15	121.18^{b} ± 28.11	132.01^{A}

Means bearing different letters within the same row, differ significantly (P< 0.05). RO : Right ovary.
LO : Left ovary

3.4. Hormonal profiles:

The effects of season of the year on testosterone concentration in FF of the dromedary she-camel were significant (P < 0.05), being higher during summer than spring, autumn and winter seasons. The highest (P<0.05) concentration of testosterone was recorded during summer and the lowest (P<0.05) concentration during spring. Similar trend was reported by Amer (2004) and Zeidan *et al.* (2008) in the dromedary camels. Heller and Ross (1979) showed that, there was a distinctive evidence of follicular steroid modulation of synthesis by androgens and a characteristic feature of the atretic follicles was high concentration of androgens in the follicular fluid. While, Abdel Ghaffar *et al.* (1995) indicated that a highly significant increase in testosterone content in FF of the atretic follicles than in normal follicular fluid in she-camel. These results might be due to the increased thickness of thecal layers which might be the source of androgens and the last layer affected the atresia process (Moor, 1977). Moreover, as atresia progressed granulosa cells were degenerated and the thecal cells were hypertrophied (Mori *et al.*, 1982). This may indicate that concentrations of the androgens in FF were positively correlated with the androgen content of thecal cells (Grant *et al.*, 1989).

In respective to ovary side, testosterone concentration in FF was highly significant (P<0.05). Testosterone hormone concentration was significantly (P<0.05) higher in the right than in the left ovary during all seasons of the year. The high androgen concentration in the follicles might not always signify atretic status, because healthy follicles might transit through an androgen dominant phase before acquiring the capacity to synthesize increased amount of estrogen (Mc Natty *et al.*, 1984).

The effects of season of the year on oestradiol 17-β concentration in FF in the dromedary she-camel were significant (P<0.05), being higher during winter and spring than autumn and summer seasons. The highest (P<0.01) value of the FF estradiol-17β concentration was recorded during winter and the lowest (P<0.01) value during summer. These results are in agreement with those of Agarwal *et al.* (1987) who found that the oestradiol 17-β concentration elevated during breeding (winter) and decreased during non-breeding season (summer). Also, Abd El-Azim (1996) showed that the highest level of oestradiol-17β was recorded in winter (130 pg/ml) and spring (117.42 pg/ml) and the lowest level in autumn (83.60 pg/ml) and summer (54.58-pg/ml). These results may be attributed to the involvement of estrogens in modulation of sexual behavior (McEwen, 1976) and testosterone secretion (Eiler and Graves, 1977) in the male one-humped camel. It is hypothesized that decreasing light hours and probably low temperature might be instrumental in triggering the hypothalamic hypophysial axis as was observed in other short day breeders like sheep (Turek and Campbell, 1979). In addition, Bedrak *et al.* (1983) observed that the relative activity of several enzymes associated with testosterone and its conversion to estrogen in the blood plasma of male one-humped dromedary camel was significantly lower during the non mating season than that of the mating one.

In respect to ovary side, oestradiol 17-β concentration was significantly (P < 0.05) higher in the left than in the right ovary during all seasons of the year. These results are in agreement with those of Amer (2004) and Zeidan *et al.* (2008).

Second experiment:

In vitro maturation:

The effects of breeding and non-breeding seasons with the different maturation media on cumulus expansion and nuclear maturation of the dromedary camel oocytes are shown in Table 5 and Plates 1 , 2, 3 and 4.

Our results revealed highly significant (P< 0.05) increase in the number of oocytes collected during breeding season, that showed postmaturation cumulus expansion (Plate 1), meiosis metaphase I (Plate 3), metaphase II (Plate 4) and Plates 3 and 4 after cultivation of the oocytes. While, Plate 2 shows oocytes at germinal vesicle (GV) stage. Regarding the total rate of expansion of cumulus complexes after 24 hours of maturation, it was significantly (P<0.05) higher in breeding season (80.7%) compared to the non-breeding season (66.5%). The incidence of oocytes reached MI and MII stages of nuclear maturation was significantly (P<0.05) higher in breeding season (20.7and 60.0 %, respectively) in comparison to the non-breeding season (27.0 and 39.5 %, respectively). These results are in agreement with those of Amer *et al.* (2003) who confirmed that, the rate -of nuclear maturation increased from 12 to 24 hrs and remained constant up to 36 hrs and the rates of metaphase-II oocytes were higher during breeding season than non-breeding one in dromedary camel.

Cultivation of the oocytes in TCM-199 showed cumulus expansion , MI and MII being 85.2, 17.0 and 68.2% compared with 69.4, 16.7 and 52.8 % in oocytes cultured in Basal medium and 87.5, 33.9 and 53.6 %, respectively in Ham's F-10 while, that were 81.3, 18.8 and 62.5%, respectively for oocytes cultured in Hank's medium. On the other hand, in non-breeding season the values were 66.7, 22.2 and 44.4%, 60.0, 20.0 and 40.0%, 75.0, 23.3 and 51.7 % and 60.0, 40.0 and 20.0% for TCM-199, Basal, Ham's F-10 and Hank's media, respectively. The highest (P < 0.05) rate of the non-matured oocytes was recorded with the Basal medium during breeding season and Basal and

Hank's media during the non-breeding season. Meanwhile, the non-matured oocytes rate was significantly ($P < 0.05$) higher during the non-breeding season than breeding one. Similar trends were reported by Amer (2004) and El-Harairy *et al.* (2006) in the dromedary camel.

When the type of culture media related to the rate of oocyte maturation, there was no differences in cumulus expansion except with Basal medium which produce the lowest ($P < 0.05$) incidence in breeding season and Basal and Hank's media in the non-breeding season. With all types of media, the rate of cumulus expansion and M II oocytes was significantly ($P < 0.05$) higher in breeding than non-breeding season. In breeding season, TCM-199 and Hank's media showed the highest ($P < 0.05$) rate of M II oocytes, whereas, Ham's F-10 medium showed the highest ($P < 0.05$) rate of MI oocytes. In non-breeding season, TCM-199 and Ham's F-10 showed the highest ($P < 0.05$) rates of M II oocytes, while, Hank's medium showed the highest ($P < 0.05$) rate of MI oocytes. The oocytes were cultivated for 36 hours which was the common period for cultivation of camel oocytes. Abdoon, (2001) cultured of camel oocytes for 36 hours produced higher ($P<0.01$) percentages of cumulus expansion and oocytes at MII. Similar trends were reported by Amer *et al.* (2003), Amer (2004) and Khalil (2009) in the dromedary camels.

4. Conclusion:

In conclusion, camel (*Camulm dromedaries*) showed better, follicular fluid components and oocyte status during breeding season (short daylight) than the non-breeding season (long day light). Ovarian activity showed higher in the left than right ovary. Acceptable dromedary camel oocytes maturation rate at metaphase II stage was obtained when oocytes were aspirated form the left ovary and cultured in TCM-199 or Ham's F-10 maturation media. Thus, season and maturation media had important role in improve the *in vitro* fertilization (*IVF*) and embryo transfer programs to enhance the fertilizing ability of camel. Therefore, further detailed investigations are needed to open away to improve the embryo production and transfer programs in the dromedary camel during both breeding, as well as, non-breeding season.

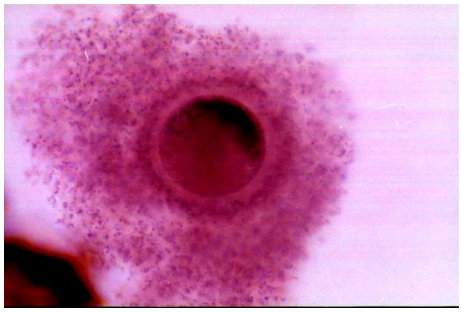

Plate. 1: Oocyte shows postmaturation cumulus expansion (x300).

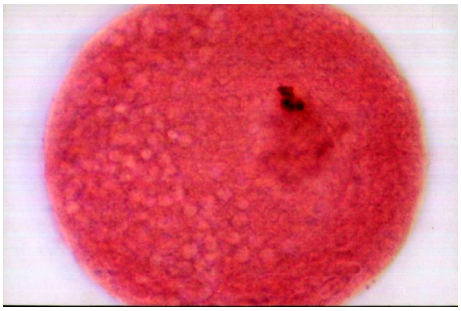

Plate. 2: Oocyte showing G V stage stained by Orcein stain (x1200).

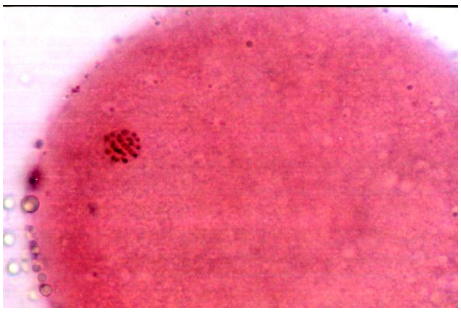

Plate. 3: Oocyte showing MI stained by Orcein stain (x1200).

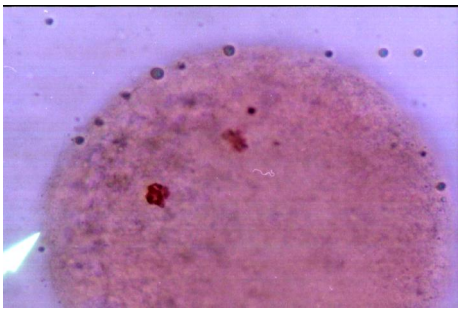

Plate 4: Oocytes showed MII stained by Orcein stain (x1200).

Table (5) : Percentage of cumulus expansion and nuclear maturation of the camel oocytes during breeding and non-breeding seasons with the different maturation media.

Maturation media	Breeding season					Non-breeding season				
	No. of oocytes	Matured oocytes			Non-matured oocytes	No. of oocytes	Matured oocytes			Non-matured oocytes
		Cumulus expansion	Metaphase I	Metaphase II			Cumulus expansion	Metaphase I	Metaphase II	
TCM-199 (%)	88	75	15	60	13	45	30	10	20	15
		(85.2)^{a}	(17.0)^{b}	(68.2)^{a}	(14.8)^{b}		(66.7)^{b}	(22.2)^{b}	(44.4)^{bc}	(33.3)^{b}
Basal medium (%)	72	50	12	38	22	30	18	6	12	12
		(69.4)^{b}	(16.7)^{b}	(52.8)^{b}	(30.6)^{a}		(60.0)^{b}	(20.0)^{b}	(40.0)^{c}	(40.0)^{a}
Ham's F-10 (%)	56	49	19	30	7	60	45	14	31	15
		(87.5)^{a}	(33.9)^{a}	(53.6)^{b}	(12.5)^{b}		(75.0)^{a}	(23.3)^{b}	(51.7)^{a}	(25.0)^{c}
Hank's medium (%)	64	52	12	40	12	50	30	20	10	20
		(81.3)^{a}	(18.8)^{b}	(62.5)^{a}	(18.8)^{b}		(60.0)^{b}	(40.0)^{a}	(20.0)^{d}	(40.0)^{a}
Means (%)	280	226	58	168	54	185	123	50	73	62
		(80.7)^{A}	(20.7)^{A}	(60.0)^{A}	(19.3)^{B}		(66.5)^{B}	(27.0)^{A}	(39.5)^{B}	(33.5)^{A}

a, b, c, d : Means bearing different letters within the same column, differ significantly ($P < 0.05$).
A, B : Means bearing different letters within the same row, differ significantly ($P < 0.05$).

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Synthesis And Evaluation Of Novel Cationic Monomers Viscosifiers For Oil Well Drilling Fluids

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Abstract: Novel cationic monomers capable of forming viscoelastic fluid were prepared. The monomers were formed through the quternzation reaction of allyl halides with dimethylalkylamines, triethanolamine or N-N dimethyl aniline. The chemical structures of the prepared monomers were conformed using FTIR and H^1NMR spectroscopy. The result of the spectroscopic analysis indicate that they were prepared through right method they have high purity and there surface properties were studied. The cationic monomer products were evaluated as viscosifiers and filter loss additives for water –base mud because they were capable of forming viscoelastic fluids in high brine solution. Rheological properties, gel strength, filter loss and thermal stability of the water- based mud formulated with the new cationic monomers were studied compared to the commercial viscosifier (reference sample mud). [A.M., Badwi, M. M., Dardir and H. M., SYNTHESIS and EVALUATION of NOVEL CATIONIC MONOMERS VISCOSIFIERS for OIL WELL DRILLING FLUIDS. Journal of American Science 2011;7(1):473-484]. (ISSN: 1545-1003). http://www.americanscience.org.

Keyword: Drilling fluids-Viscosifier-Rheological properties

Introduction:

In the field of drilling for the exploration for oil and gas, an important component is that of the formulation of drilling muds. [1-5] Drilling muds are the fluids which are used to maintain pressure, cool drill bits and lift cuttings from the holes and vary in composition over a wide spectrum. Generally, drilling muds are based on aqueous formation or oil-based formulations. [6-12] A conventional water-based drilling mud formulation is composed basically of the following ingredients: water, clay such as bentonite, lignosulfonate, a weighting agent such as $BaSO_4$ (Barite) and a caustic material such as sodium hydroxide to adjust the pH of the drilling mud to a pH of about 10 to 10.5 . [13-15] This formulation with its high density due to the addition of high concentrations of insoluble solid, high density particulates (weighting agents such as Barites). However, this particulates inhibited the drilling rate and possibly damaged a variety of underground formations. [16-18] This problem becomes even more acute as the drilling fines are introduced into the mud. Therefore, there has been a substational need for a homogenous, high density drilling mud which exhibits good performance at both high temperature and high ionics strength. Previously, a very desirable change in the formulation of a drilling fluid would be by the elimination of all added particulates.[19] One particular approach to this problem is to formulate a drilling fluid which is clear, homogenous, dense, single phase and possess the appropriate viscosity requirements. Therefore a water-based mud containing principally clay and a polymeric viscosifier in a high concentration brine (weighting agent) could meet the above-stated requirements.

Polymeric materials are generally considered useful as viscosification agents when dissolved in appropriate solvent system. The major reason for this viscosity enhancement is due to the very large large dimensions of the individual polymer chain as compared to the dimension of the single solvent molecule. Any increase in the size of the polymer chain will produce acorres ponding enhancement in viscosity of the solution. This effect is maximized when the polymer is dissolved in a good solvent. Therefore, in general, a soluble polymer is useful for thickening solvent while a water soluble polymer is appropriate for increasing the viscosity of aqueous systems. With regarded to aqueous solutions, solvent soluble nonionic polymers and high charge density sulfonated or carboxylate polyelectrolyts are quite useful in this regard and are commonly used materials. These material become especially effective at concentration where the individual polymer chains begin to overlap.[20] To overcome the difficulties experienced in conventional polymer viscosifiers and rheological control additives in aqueous media a novel family of cationic-alkyl monomers i.e. polymerizeable moieties, form a large structure in solution, and enables the efficient viscosification of aqueous fluids without the need for a moderate or high molecular weight water soluble polymer. The structure of these monomers are useful and very effectives viscosifier for aqueous solution. In addition, these monomers have markedly unique and improved solution properties as compared to the conventional water soluble polymers. These monomers overcomes the difficulties experienced in conventional polymeries viscosifiers and rheological control additives in aqueous media. In particular, it enables the efficient viscosification of aqueous fluids

without the need for a moderate or high molecular weight water soluble polymer.

The main property of the prepared monomers are being a cationic surfactant. So, they achieve solubility and thickening efficiency which make this system very sensitive, as will as very sensitive to small changes in surfactant and polymer concentrations.[21-22]

Experiments:

Synthesis of monomer structures:

Synthesis of quaternary ammonium compounds:

N,N dimethyl dodecylamine, triethylamine or N,N dimethyl aniline (1 mole) each was added to allyl Bromide (1 mole) in the presence of xylne as a solvent, the reaction mixture was refluxed at 50°C for 6 hours. The solution was evaporated under reduced pressure and the monomers were further purified through conventional analytical techniques.(23)

1- Correct elemental analysis was measured in Micro Analytical Center, Cairo University.

1- FTIR spectra using ATI Mattsonm infinity seriesTM , Bench top 961 controlled by win first TM V2.01 soft ware. (Egyptian Petroleum Research Institute).

2- H^1NMR was measured in DMSO-d6 by spect varian, GEMINI 200 (1H200 MHz). (Micro Analytical center, Cairo University).

Elemental analysis, FTIR and H^1NMR analysis confirm that the monomers are very pure and have the following molecular structure

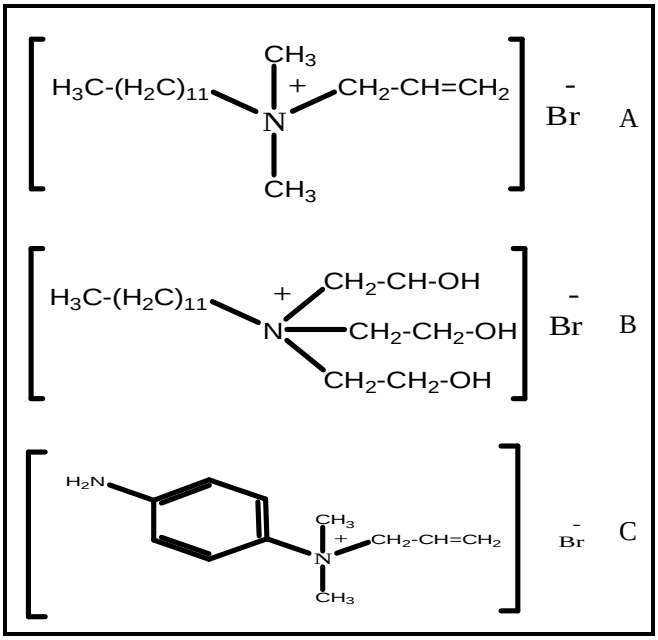

Fig (1) the chemical structures of the synthesized compounds.

Surface tension and interfacial tension:

Surface tension and interfacial tension of the prepared compounds solutions were measured using Du-Nouy tensiometer (Krass type 8451). The interfacial tension between 0.1% surfactant solution and light paraffin oil was also measured at 25°C.

The surface parameters of the synthesized compounds:

Critical micelle concentration (CMC):

The values of CMC of the prepared compounds were determined using surface tension techniques, where in this method, values of the surface tension measurements were plotted against the corresponding concentration. The interrupt change in the SC curves express on the CMC concentrations.

Effectiveness (πCMC):

π CMC is the difference between the surface tension of the pure water (γ_0) and the surface tension of the surfactant solution (γ) at the critical micelle concentration.

$$\pi CMC = \gamma_0 - \gamma$$

Efficiency (PC_{20}):

Efficiency (PC_{20}) is determined by the concentration (Mol/L) of the surfactant solutions capable to suppress the surface tension by 20 dyne/cm.

Maximum surface excess r_{max}:

The values of the maximum surface excess r_{max} were calculated from surface or interfacial data by the use of Gibbs equation.

$$r_{max} = -1/2.303RT(\delta\gamma/\delta\log c)_T$$

Where:

r_{max} = maximum surface excess in mole/cm^2.

R = Univeral gas constant 8.31 x 10^7 Ergs mol^{-1}

T = absolute temperature (273.2 + °C)

$\delta\gamma$ = surface pressure in dyne/cm.

C = surfactant conc.

($(\partial\gamma/\gamma\log c)_T$ is the slope of a plot surface tension Vs. concentration curves below CMC at constant temperature.

Minimum surface area (A_{min}):

The area per molecule at interface provides information on the degree of packing and the orientation of the adsorbed surfactant molecule. The average area (in square angstrom) occupied by each molecule adsorbed on the interface is given by:

$$A_{min} = 10^{16}/\Gamma_{max}N$$

Where:

r_{max} = maximum surface excess in mole/cm^2

N = Avogadro's number $6.023x10^{23}$

Tests for water – base mud:

The prepared cationic monomers A,B and C were evaluated as a viscosifier and filter loss additives for water-base mud compared to the commercial viscosities. The mud batches of local bentonite using fresh water treated by a new prepared cationic monomers A, B and C or reference viscosifier R with concentration of 0.5% for a viscosifier additive . [23]

Mud formulation:

Formulation of the mud were as follow:

6% of local bentonite + 500 ml fresh water

1- The samples were mixed in a Hamiltan mixer for 20 minutes then cured overnight.
2- Each sample was stirred for 15 minutes, before the rheological and filtration properties were measured before adding viscosifier.
3- 0.5% of a new viscosifiers A,B and C and commercial one were added to local bentonite mud batches.
4- The samples were mixed for 20 minutes and cured overnight.
5- Each sample was stirred for 15 minutes, then the rheological and filtration properties were measured.

MR: Water-base mud formulated of 6% local bentonite and (commercial emulsifier R).

MA: Water-base mud formulated of 6% local bentonite and 0.5% cationic monomer (A).

MB: Water-based mud formulated of 6% local bentonite and 0.5% cationic monomer (B).

MC: water-based mud formulated of 6% local bentonite and a cationic monomer (C).

Results and discussion:

Chemical structure:

The chemical structure of the prepared cationic monomers were confirmed by:

1- Correct elemental analysis.
2- The FTIR spectroscopy was used to identify the functional groups of the prepared cationic surfactant .The FTIR spectrum of compound (A) shows, stretching vibration of –C-H aliphatic symmetric and asymmetric at 2827.15 & 2976.18 cm^{-1} respectively, 2605.64cm^{-1} for (N^+), (C – N) amine at 1034.58 cm^{-1}, and weak absorption band of (C=C) alkene at 1630.88cm^{-1}, and $–CH_2$ bending at 1438.19cm^{-1}. we observe disappearance of the absorption bonds of –NH at 3300cm^{-1}.

The FTIR spectrum of compound (B) shows, (C=O) stretching at 1038.88cm^{-1}, (C-N) amines at 1302.80cm^{-1}, ($-CH_2$) bending at 1455.18cm^{-1}, weak absorption band of (C=C) alkene at 1594.36 cm^{-1}, (CH_2) stretching at 2924.29 cm^{-1} and broad absorption band of (O-H) at 3300cm^{-1}.

The FTIR spectrum of compound (C) shows, (C-H) aromatic out-of-plane bend at 690cm^{-1}, (C-N) amine at 1120.92cm^{-1}, medium absorption band of (C=C) aromatic at 1487.90cm^{-1}, weak absorption band of (C=C) alkene at 1627.45cm^{-1}, (C-H) Aromatic stretching at 3114.79cm^{-1} and absorption band of (NH_2) stretching at 3422.04cm^{-1}.

The H^1NMR spectra data (δppm) of the compound (A), shows bands at 0.787 – 0.848 ppm (t, 3H, $C\underline{H}_3(CH_2)_{11}$ –), at 1.137-1.212ppm (m,4H, $CH_3(C\underline{H}_2)10$-), at 2.129.2.218ppm (t,3H,CH_3 $(CH_2)10$ $(C\underline{H}_2)$ -), at 3.060ppm (S, 1H, $\underline{H}_3$ C – N – $C\underline{H}_3$), at 4.075 – 4.111ppm (d, 2H, - N – $C\underline{H}_2$ – CH = CH_2), at 5.595 – 5.692ppm (d, 2H, $C\underline{H}_2$ = CH -), and at 5.968 – 6.173ppm (m, 5H, CH_2 = C<u>H</u> – CH_2 –).

The H^1 NMR spectra data of compound (B), shows bands at 3.456 – 3.505ppm. (t, (3 H– N– ($C\underline{H}_2$ – CH_2)$_3$) ,at 3.838 ppm (s, 1 H– (CH_2– O<u>H</u>)$_3$) ,at 3.635 – 3.688 ppm (t, 3 H– (H_2C –$C\underline{H}_2$ – OH)$_3$) ,at 3.635 – 3.688 ppm (d, 2H = HC – $C\underline{H}_2$ – N), at 5.559 – 5.700ppm (d, 2<u>H</u> – CH = CH_2), and at 5.987 – 6.156ppm (m, 5H CH_2 = C<u>H</u> – CH_2 – N).

The H^1NMR spectra of comp. (C) shows bands at 3.676ppm (S, 1H, $\underline{H}_3C$ – N – $C\underline{H}_3$), at 3.874 – 3.906ppm (d, 2H, = CH – $C\underline{H}_2$ – N), at 4.124 – 4.162ppm (d, 2H, - CH = $C\underline{H}_2$), at 4.669 – 4.696ppm (S, 1H, $\underline{H}_2N$ – ph – N -), at 5.446 – 5.582ppm (m, 5H, CH_2 – C<u>H</u> = CH_2), at 7.528 – 7.555ppm (2H, - <u>Ph</u> – N^+H_2), and at 7.964 – 8.059ppm (2H, - <u>ph</u> – N -).

Surface active properties:

Surface and interfacial tension:

a) The surface tension:

Surface tension values were measured for aqueous solutions of the prepared cationic monomers surfactant A,B and C with different concentration at room temperature and the data are represented in surface tension conc. curves Fig. (2). It is clear that surface tension decrease by increasing concentration and also decrease in A than B than C. This is due to in compound (A) long straight chain (hydrophobic chain) which has higher repulsion forces in the water medium. Hence, the molecules will tend to adsorb at the interface with high concentration. Meanwhile, in compound (B) the shorter branched chain has lower tendency to adsorb at the interface due to the lower repulsion occurred from the aqueous phase.

The compound (C) has one benzene ring so that the hydrophobicity of the molecules decreases and

become more hydrophilic which facilitate the molecules to found in the bulk of their solutions and hence the surface tension stays at higher values.

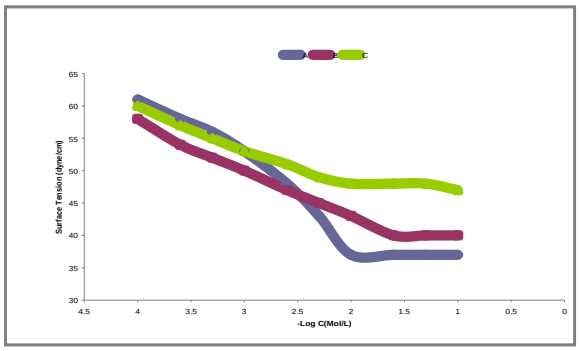

Fig.2 Variation of the surface tension with logarithm of the prepared cationic surfactants concentrations in water at 25°C

We can explain the decrease of surface tension by increasing concentration that increasing length of the hydrophobic chain is due to adsorption of surfactant molecule at the interface. We know that when materials that containing hydrophilic and hydrophobic group attached together in the same molecule dissolved in a solvent, it distort the structure of the solvent and therefore increase free energy of the system. Thus the molecules concentrate at interface in a way to minimize the free energy of the system, where the hydrophobic part oriented away from the solvent to avoid energically unfavorable contact with aqueous media, and the hydrophilic group is directed toward the bulk. This adsorption at interface provide an expanding force acting against the normal surface tension, thus surface tension decrease. [25,26] So by increasing the surfactant concentration, the adsorptions at interface will increase so surface tension decreases until a stable lower level is achieved. This lower level of surface tension corresponds to maximum monolayer level adsorption at air-water interface. Further increasing surfactant concentration above this maximum interfacial adsorption level lead to formation of surfactant aggregation known as micelle [27] in solution.

b) Interfacial tension:

The interfacial tension between 0.1% surfactant solution and light paraffin oil at 25°C were measured and data are shown in the table (1). From these data it is observed that by increasing hydrophobic chain length of the prepared cationic surfactants, the interfacial tension decrease. [28, 29]

Table (1). Surface tension and interfacial tension of the synthesized cationic surfactants.

Surfactant	Surface tension, mN/m	Interfacial tension, mN/m
A	37	12
B	40	23
C	46	29

The surface parameters

The critical miclle concentration (CMC):

CMC values of the prepared cationic surfactant were determined by plotting the surface tension (γ) of surfactant solutions versus their bulk concentration in mole/liter at room temp. the CMC values are listed in table (2) showing a decrease in the CMC with (A < B < C) due to increasing in the length of the hydrophobic chains [30] .

Effectiveness (πCMC):

πCMC values are listed in table (2). It appears found that, effectiveness increases with the increasing of hydrophobic chain length [31].

Maximum surface excess (Γ_{max}):

The values of Γ_{max} are represented in table (2). It is noted that increasing the hydrophobic moiety length of the prepared surfactants, Γ_{max} shift to lower concentration and thus the surfactant molecules are directed to the interface which decrease the surface energy of their solutions [32,33] .

Minimum surface area (A_{min}):

The minimum area per molecule at the aqueous solutions/ air interface for the prepared surfactants is listed in table (2). It is clear that A_{min} increase with increasing length of hydrophobic moiety due to decreasing Γ_{max} thus the distance between molecules are increased and correspondingly A_{min} increases [34].

Efficiency (PC 20):

Efficiency values of the prepared cationic surfactants are given in the table (2). From these data it is observed that increasing the alkyl chain length, the efficiency decrease. This is due to the fact that the efficiency of adsorption at interfaces increase linearly with an increase in the carbon atoms in hydrophobic group as illustrated in discussion surface tension [25-28].

Table (2). Surface properties of the synthesized cationic surfactants at 25°C

Surfactant	CMC	γ_{CMC}, mN/m	Pc_{20}	$X10^{-100}_{max}$, mol.cm^{-2}	A_{min} nm
A	0.005	35	0.00098	1.00295	1.6554
B	0.02	32	0.0003	1.13106	1.467
C	0.07	26	0.00025	2.20207	0.753

Thermodynamic parameters:

Adsorption and micellization processes of surfactant molecules are considered as phase transformation either from singly state molecule in the solution into adsorbed molecules at the interface (adsorption) or into the well aggregated molecules in the form of micelles (micellization). The functions are calculated using Gibb's adsorption rules As follows:

For micellizarion

$$\Delta G_{mic} = RT\,In\,(CMC)$$

For adsorption

$$\Delta G_{ads} = \Delta G_{mic} - 6.023\times10^{-1}\times\pi_{CMC}\times A_{min}$$

Standard free energies of micellization and adsorption for the prepared surfactants are calculated at (25°C) according to Gibb's equations of thermodynamics and their values are listed in tables (3).

Table (3). Thermodynamic parameters of the synthesized cationic surfactants at 25°C

Surfactant	G_{mic}, Kj/mol	G_{ads}, Kj/mol.
A	-26.2244	-26.234
B	-19.362	-19.393
C	-13.162	-13.202

Negative values of the standard free energies of both micellization and adsorption for the prepared surfactants indicate that the micellization and adsorption are spontaneous processes.

The spontaneously of the process is contributed to the repulsion between the different hydrophobic moieties and the polar solvent. Hence, by increasing the hydrophobic chain length (in other statement, HLB value) increases the tendency of these molecules towards adsorption increases which result in increased negativity of ΔG_{ads} values. Thus ΔG_{mic} and ΔG_{ads} become more negative with increasing chain length. [35,36]

Evaluation of the synthesized cationic monomers as a viscosifier and water-loss control agents for water-based mud: The prepared cationic surfactants A, B and C were evaluated as viscosifiers and filter loss additives in water-base mud. The mud formulation contain local bentonite (6%) and (0.5%) of the new viscosifier compared to the mud formulation with imported viscosifier (R).

Rheological properties: At 60°F the rheological result illustrated in fig (3) shows that the apparent viscosity for water-base mud treated with cationic surfactant A,B and C were 27, 26, 24 (cp) for MA, MB and Mc, respectively and the apparent viscosity of the reference sample MR is 25 (cp). The plastic viscosity changed from 19(cp) to 17 (cp) for MA, MB and Mc muds compared to the MR which was 16(cp).

The yield point for MA, MB and MC were (16, 18, 12 1b/100ft^2), respectively while for MR was (16 1b/100ft^2).

From the above result we can conclude that water-base muds MA and MB exhibit rheological properties better than MR while MC mud has rheological properties less than MR.

Gel strength: fig (4) illustrates the result of the gel strength of water-base muds MA, MB and MC compared to the MR at 60°F.

G 10sec.: the gel strength were (13, 15, 10 1b/100ft^2) for MA, MB and MC, respectively while for MR was (13 1b/100ft^2) which is compatible with gel strength of MA.

G 10mints: the gel strength were (16 and 17 1b/100ft^2) for MA and MB which were more than (16 1b/100ft^2)for MR . MC exhibited a gel strength less than MR (11 1b/100ft^2).

Thixotropy: for MA (3 1b/100ft^2) was more than that of MR (2 1b/100ft^2). For MB (2 1b/100ft^2) and MC (1 1b/100ft^2) were less than MR. from the above result we can conclude that all the muds were stable and can keep their rheological properties for a period of time during the drilling operation without change.

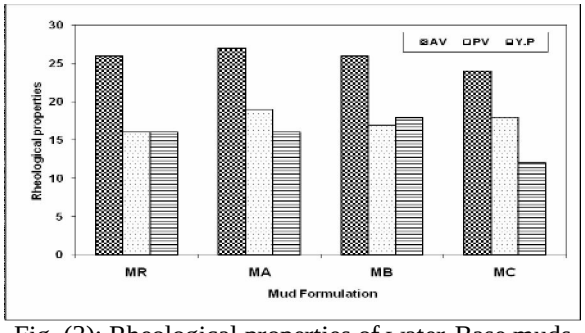

Fig. (3): Rheological properties of water-Base muds formulated with newly prepared viscosifiers compared to the reference sample mud

Effect of temperature on rheological properties of water – base mud:

In our study, the rheological properties changes with increasing temperature ranging between

60°F and 200°F. For local bentonite treated with imported viscosifier (MR): from the data represented in fig (5), it is show that the apparent viscosity decrease from 25(cp) to 14(cp), plastic viscosity decreases from 16(CP) to 10(CP) and the yield point changes from (16 1b/100ft^2) to (9 1b/100ft^2).

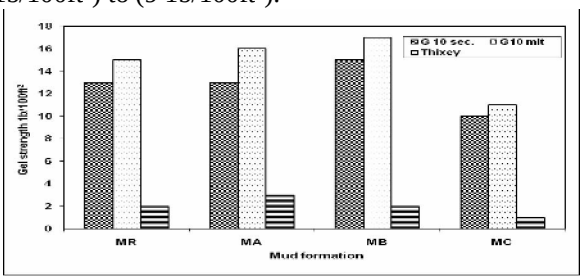

Fig. (4): Gel strength of water-Base mud formulated with newly prepared viscosifiers compared to the reference sample mud

Local bentonite treated by new viscosifiers:

MA: The apparent viscosity decreased from 27(CP) to 16(CP), the plastic viscosity decreased from 19(CP) to 12(CP) and the yield point changed from (16 1b/100ft^2) to (10 1b/100ft^2).

MB: The apparent viscosity changed from 26(CP) to 14(CP), the plastic viscosity changed from 16(CP) to 10(CP) and yield point decreased from (18 1b/100ft^2) to (8 1b/100ft^2).

MC: The apparent viscosity changed from 24(CP) to 12(CP), the plastic viscosity decreased from 18(CP) to 11(CP), yield point changed from (12 1b/100ft^2) to (5 1b/100ft^2).

Results of the Rheology-temperature relations indicate that the new additives A and B satisfy the minimum requirements for API specification while C has less results compared to imported vis-cosifier R.

Effect of temperature on gel strength.

Local bentonite with imported viscosifier: fig. (6) reveals that the gel strength decreases from (13 1b/100ft^2) after 10 second to (81b/100ft^2) as the temperature raised for 60°F to 200°F. Also it decreased for (15 1b/100ft^2) to (8 1b/100ft^2) after 10 mints.

Local bentonite treated by new viscosifiers (as the temperature raised from 6o°F to 200°F).

MA: The gel strength after 10 sec. decreased from (13 1b/100ft^2) to (8 1b/100ft^2) and after 10 mints. decreased from (13 1b/100ft^2)to (7 1b/100ft^2).

MB: The gel strength decreased from (16 1b/100ft^2) to (8 1b/100ft^2) for G10 sec. and it decreased from (16 1b/100ft^2) to (7 1b/100ft^2) for G10 mint.

MC: The gel strength changed from (10 1b/100ft^2) to (4 1b/100ft^2) after 10 sec and it changed from (10 1b/100ft^2) after 10 min.

The results showed that the imported viscosifier R and new prepared viscosifiers A and B are compatible with the requirements of field mud additives but the new prepared viscosifier C has less compatibility.

Effect of temperature on shear rate and shear stress:

The shear stress value decreases as the shear rate decreases at the same temperature and fig. (7) reveals that:

At 60°F, the shear stress value decreased from (55 1b/100ft^2) to (12 1b/100ft^2) for MA. From (52 1b/100ft^2) to (15 1b/100ft^2) for (MB) and from (49 1b/100ft^2) to (10 1b/100ft^2) for (MC) whereas it decreased from (46 1b/100ft^2) to (13 1b/100ft^2) for reference (MR).

At 120°F, the values decreased from (45 1b/100ft^2) to (11 1b/100ft^2) for MA. From (43 1b/100ft^2) to (11 1b/100ft^2) for MB and from (40 1b/100ft^2) to (9 1b/100ft^2) for MC whereas it decreased from (39 1b/100ft^2) to (9 1b/100ft^2) for MR.

At 180°F, the values decreased from (36 1b/100ft^2) to (8 1b/100ft^2) for (MA), from (30 1b/100ft^2) to (8 1b/100ft^2) for (MB) and from (30 1b/100ft^2) to (8 1b/100ft^2) for MC whereas the values of MR decreased from (30 1b/100ft^2) to (8 1b/100ft^2).

The result show that muds treated with the new viscosifiers A, B and C perform good results compared to the imported viscosifier R under varying temperature.

Effective viscosity: The effective viscosity of water-based mud MA, MB and MC that were treated with the new viscosifiers (A, B, C) decreased as the shear rate increased similar to reference mud sample (MR) that was treated with imported viscosifier R. These results are illustrated in fig. (8) where vertical lines show the rpm equivalents of shear rate in sec^{-1}. Drilling fluids are usually pseduoplastic, i-e shear thinning fluids.

Filtration: table (4) shows the filter loss at pressure (100 psi) for the water-based mud (MA, MB and MC) that were treated by the new viscosifiers A, B and C compared to the reference mud sample (MR).

Table (7): filter loss (ml) for water –base mud.

Mud	Filtrate, ml
MR	13
MA	10
MB	11
MC	12

For the new viscosifier additives mud ,the corrected filter loss were between 10 ml (MA), 11 ml (MB) and 12 ml for MC whereas for MR was 13 ml. The results of all additives satisfied the international standard and the decrease of filter loss in case of MA, MB and MC indicates the stability of additives and that they show good filter loss.

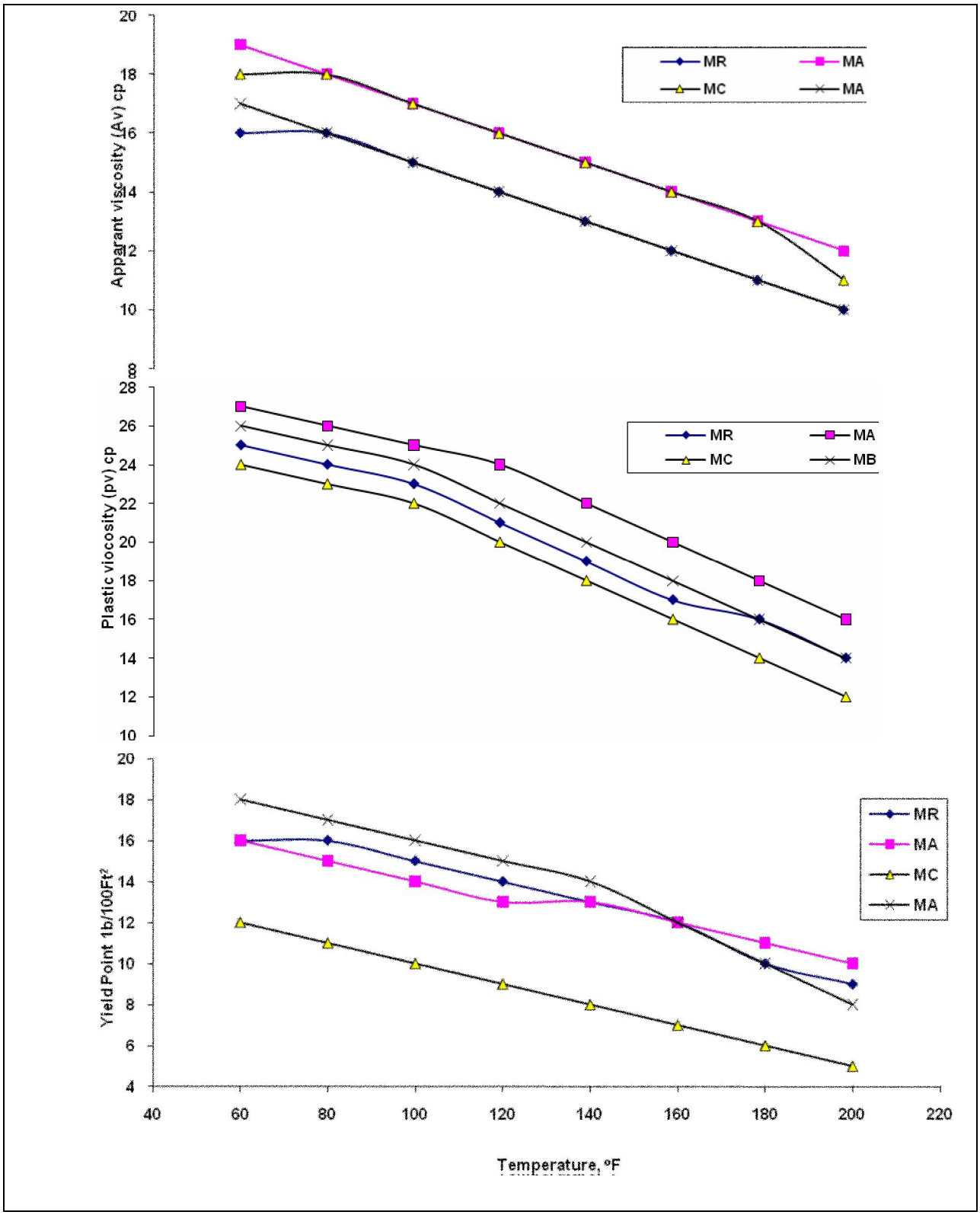

Fig. (5): -Rheological properties of water-based mud formulated with newly prepared viscosifiers with varying temperature.

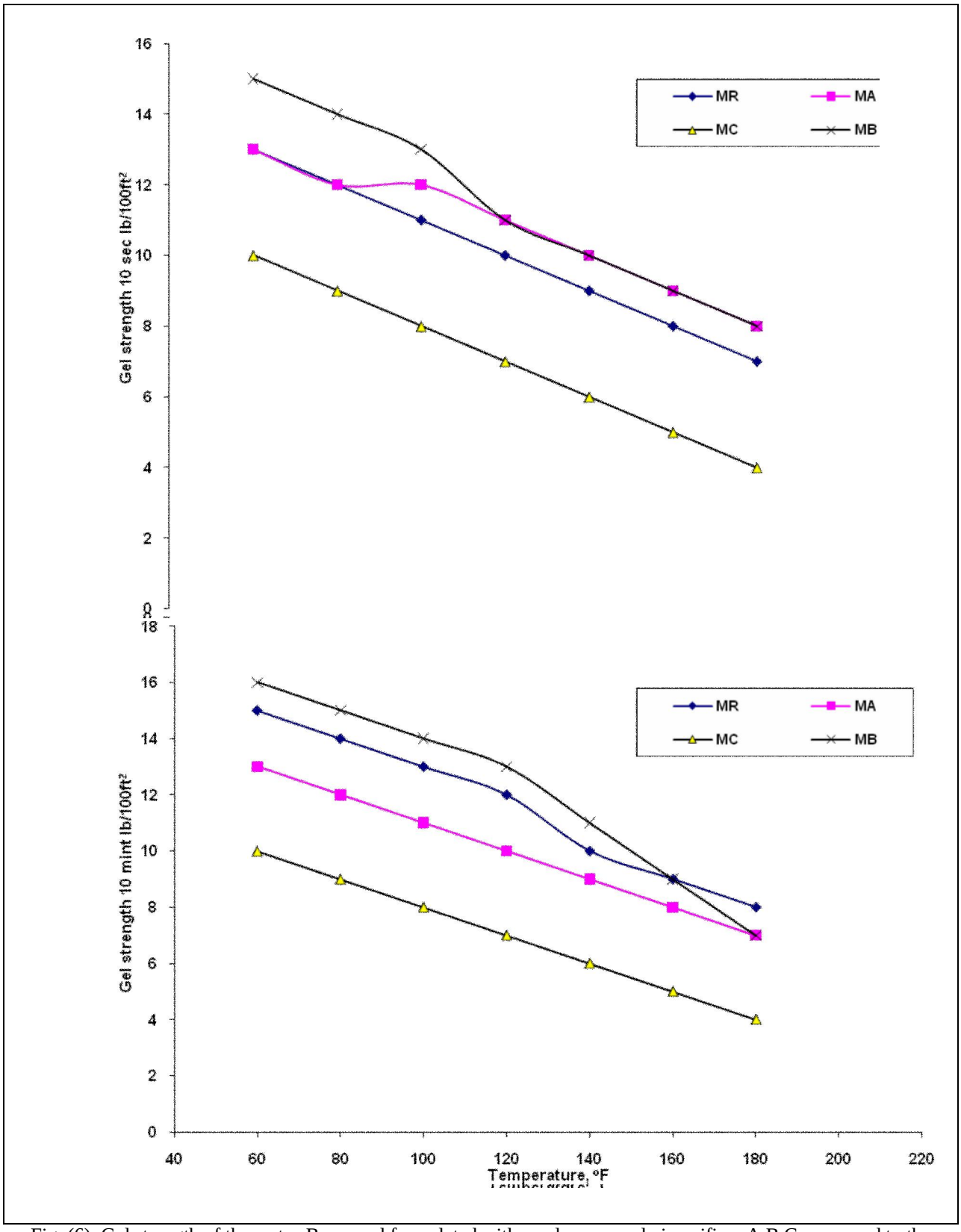

Fig. (6): Gel strength of the water-Base mud formulated with newly prepared viscosifiers A,B,C compared to the reference sample mud MR under varying Temp.

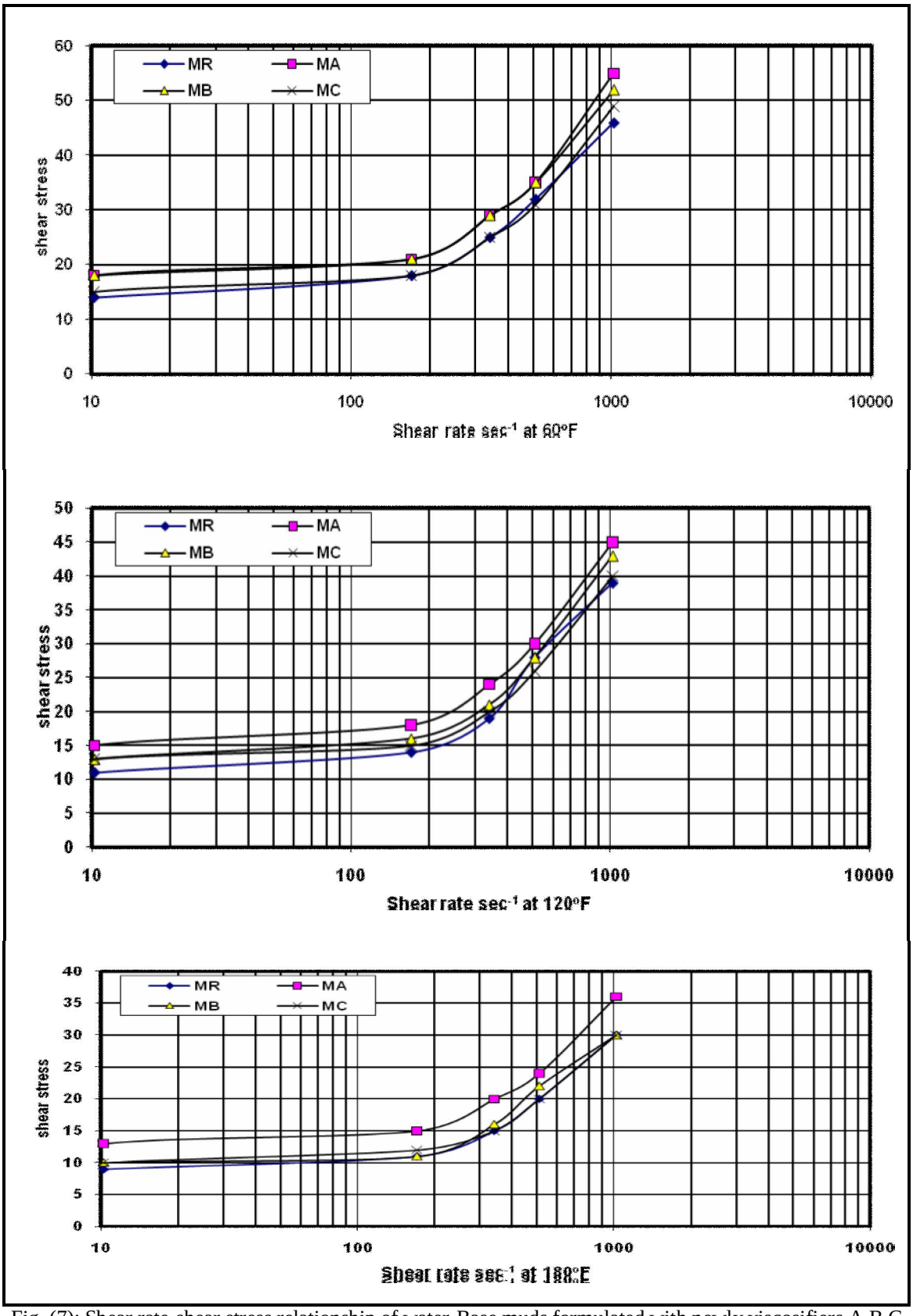

Fig. (7): Shear rate-shear stress relationship of water-Base muds formulated with newly viscosifiers A,B,C compared to reference sample mud (MR) under varying temperature

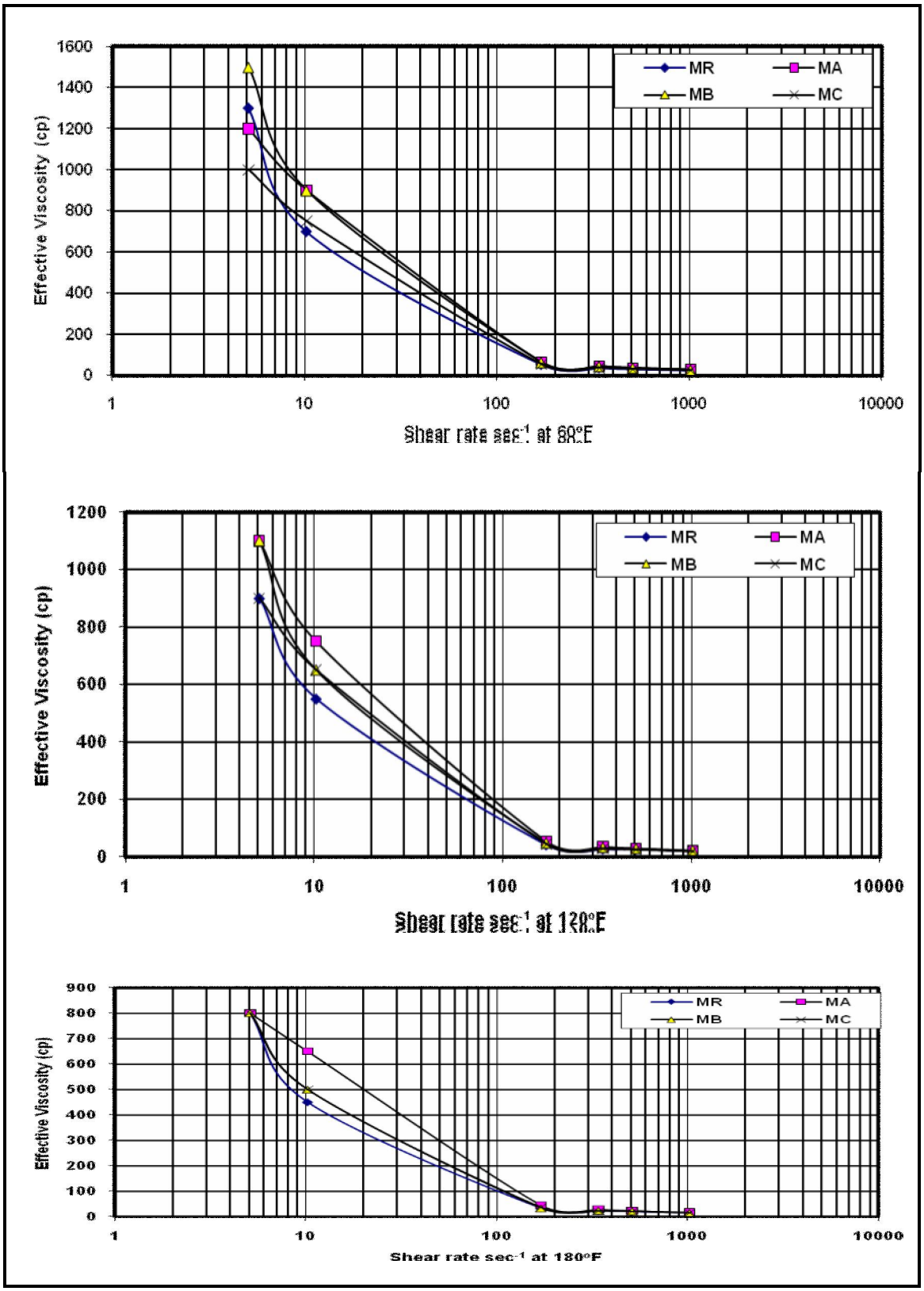

Fig. (8) Effective viscosity of water-Base muds formulated with newly prepared viscosifiers A, B, C compared to the reference sample mud MR under varying temperature

Conclusions:

From the obtained results we can conclude that:

1. All the Synthesized cationic surfactants showed good surface properties.
2. Experimental work and evaluation of the new synthesized cationic surfactants (A, B, C) show good results when utilized in the formulation of water –base mud as viscosifiers compared to the commercial viscosifier.
3. Rheological ,filtration properties of the most Synthesized viscosifiers performed a superior result compared to the commercial viscosifier

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Effect of ripening conditions on the properties of Blue cheese produced from cow's and goat's milk

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Abstract: Blue cheese (style Roquefort) was made from cow's and goat's milk. Fresh cheese was ripened at room conditions for 30 days, then resulted cheese were divided into two portions, one was complete ripened at room conditions and the other was complete ripened at refrigerator for another 30 days. Cheese samples were analyzed at 1, 30 and 60 days of ripening period, for moisture, fat, pH, total nitrogen and free amino acids. Tyrosine & Tryptophan and total volatile fatty acids contents as well as their organoleptic properties. No clear differences were observed between both goat's and cow's cheese in their gross composition. Goat's blue cheese ripened for 60 days at room temperature had a higher total free amino acids contents than that in cow's cheese, while their values were higher when cheese ripened at refrigerator than that ripened at room temperature. Blue cheese from goat's milk showed the highest total volatile fatty acids and Tyrosine & Tryptophan contents during ripening, at the end of ripening, the cheese ripened at room temperature gave the higher values than that ripened at refrigerator. Blue cheese from goat's milk ranked a higher score for organoleptic properties during ripening conditions compare with that made from cow's milk. It can be concluded that goat's milk can be successfully used in the manufacture of blue cheese and ripened at room temperature with high quality over than that from cow's milk.
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1. Introduction

The three classic "old-world" varieties of blue cheese are Roquefort (sheep's milk blue from southern France), Gorgonzola (northern Italian cow's milk blue), and Stilton (cow's milk blue from central England). The three cheeses vary quite a bit both in texture and taste. Roquefort is sweet, moist and crumbly; Stilton is firmer and spicier; and Gorgonzola (especially the variety known as *dolce*) is sweet and creamy (Deetae et al., 2007).

Blue cheese (Style Roquefort) is the main blue mould cheese produced in Egypt; this type is initially made from full cream sheep milk. During ripening of blue-veined cheese, desirable changes occur as a result of limited degradations of the carbohydrates, protein and fat of milk, (Ali, 1993). This is brought about by the combined action of milk enzymes, rennet. Starter cultures (mainly the mould) and other microbial flora, many chemical and biological interactions are involved. At the end of ripening, a complex mixture of compounds which give the nature cheese, the required balance of flavour and aroma are formed, (Varnam and Sutherland, 2001).

Proteolysis is more extensive in blue cheese than in most other varieties. Blue cheeses are characterized by the growth of Pencillium requeforti in openings within the cheese body. In addition to proteolytic enzymes originating from the milk, coagulant and starter microorganisms. The biochemistry of ripening of blue cheese is far more complex than that of internal bacterial-ripened varieties such as Cheddar or Gouda. Despite this and the international importance of a number of blue cheeses (e.g. Roquefort, Stilton and Gorgonzola) there have been relatively few studies on proteolysis in blue cheese during ripening (Zarmpoutis et al., 1996).

Proteolysis is the most important phenomenon to take place during cheese ripening. The peptides and amino acids freed by the action of proteolytic enzymes, moulds, etc. are related both to flavour intensity (McGugar et al., 1979) and to cheese age, (Ramos, et al., 1987 and Ali, 1993).

The fat and protein contents of cow's and goat's milk are generally fairly similar. However, that does not mean cow's and goat's milk are the same? Goat's milk has a more easily digestible fat and protein content than cow milk. The increased digestibility of protein is of importance to infant diets. Goat milk can successfully replace cow milk in diets of those who are allergic to cow milk. In under-developed countries, where meat consumption is low, goat milk is an important daily food source of protein, phosphate and calcium not available otherwise because of a lack of cow milk (Paul

kindstedt, 2005), so goat's milk is more suitable for producing blue cheese.

Science a long time, a great interest had been arisen to the goats all over the globe. They were considered ideal milk animals, which can convert feed of poor nutritional quality to milk. In Egypt, the total population of goats amounted to 3.4 millions. Annual goat's milk production of 376.500 tons was estimated, however only 20% was used for human consumption (EL-Abd et al., 1992).

The objective of this study was using of goat's milk in making blue cheese (style Roquefort) in comparison with cow's milk and studying the effect of ripening temperature on the properties of the resultant cheese.

2. Materials and Methods

Milk: Whole cow's milk was obtained from the herd of the Ministry of Agriculture, (Dokki, Giza, Egypt), while goat's milk was obtained from a herd of private farm in Giza district.

Starter:*Streptococcus Lactis* ssp *Lactis* (1106) was obtained from MIRCEN, Faculty of Agriculture, Ain Shams University.

Moulds:A strain of *Pencillium roqueforti* was obtained from Chr-Hansen's A/S, Horsholm, Denmark.

Rennet: Rennet powder was obtained from Chr-Hansen's, Denmark with a commercial name HA-LA.

Salt: Pure sodium chloride (NaCl) was obtained from the local market.

Table 1: Gross composition of goat's milk compared with that o cow's milk

	Cow's milk	Goat's milk
Total solids %	12.0	12.50
Fat %	3.40	3.60
Total protein %	3.45	3.20
Lactose %	4.40	4.70
Ash %	0.72	0.75
Acidity %	0.16	0.17
pH value	6.40	6.52

Average of three replicates

Data of milk composition Table(1) indicated that averages of milk TS, fat, total protein lactose, ash, acidity and pH values showed of both goat's and cow's milk.

Blue-veined cheese making:

The method of (Kosikowski, 1982) was applied. Fresh milk was heated to 73°C for 10 min., and then cooled immediately to 35°C. 1% of active *S. Lactis* was added. When the acidity of milk reached 0.22%, rennet was added (0.3g/10kg milk). The milk was left to curdle, after complete coagulation, the curd was cut into cubes (½ inch) which were settled for 5 min. and temperature was raised to 40°C. Curd was stirred occasionally during the next 30 to 60 min., until acidity reached 0.19%. 1% of salt was added and whey was drained. The curd was hooped and each layer sprinkled with *P. roqueforti* spores containing powder. The cheese was turned at intervals for several hours. Cheese was salted in 20% brine for 24 hrs. then punched. The hoops were stored in curing refrigerator at (8-10°C, 90% relative humidity). Cheese samples were taken for analysis at1, 30 and 60 days of ripening at (refrigerator and room temperature).

Methods of analysis:

The total solids, total protein, fat pH and acidity of both cow's and goat's mil were determined as given by (A.O.A.C., 1990).

The total solids, total nitrogen, fat and acidity of blue cheese were estimated according to (A.O.A.C., 1990). pH values were measured using pH-meter with a glass electrode (MV 870-Digital-pH meter). Tyrosine and tryptophan of cheese samples were measured as Vakaleries and Price (1959). Total volatile fatty acids (TVFA) of cheese samples were estimated as described by (Kosikowski, 1982).

Amino acids analysis:

Amino acids composition of cheese samples were determined according to method of Millipore Cooperative (1987) using high pressure liquid chromatographic analysis (HPLC).

Organoleptic assessment:

Blue cheese (style Roquefort) samples were scored for organoleptic properties by a taste panel of 11 persons for National Research Centre staff as described by EL-Shazly et al., (1994). The panelists scored the cheese flavour (out of 40 points) texture (out of 20 points) and colour (out of 40 points).

3. Results and Discussion

The mean values for moisture, fat, fat/DM, nitrogen and pH in blue cheese (style Roquefort) during ripening are summarized in Table (2). Moisture content decreased gradually during ripening. Its content, of goat's blue cheese was less than that of cow's cheese at fresh and during ripening

period, this may be due to the differences in initial total solids contents of both cheeses, which related to the variation of water holding capacity of both curds during cheese making. This observation may be explained by that casein micelles of goat's milk are smaller than that of cow's milk (Juarez and Ramos, 1980 and Riel, 1985). However, moisture content of cheese ripened at refrigerator was less than that ripened at room temperature at the end of ripening period. The pH of fresh blue goat's cheese was 5.20 but increased to 5.40 for cheese ripened at room temperature and 5.50 for that ripened at refrigerator, while pH value of fresh blue cow's milk was 5.30 increased to 5.60 for cheese ripened at room temperature and 5.65 for that ripened at refrigerator. The increase in the pH of blue cheese during ripening is due to the deamination of amino acids with the production of NH3 and the metabolism of lactic acid to CO2. Similar results have been reported by (Zarmpoutis et al., 1996). Also, these results were agreed with (Abd-EL-Salam et al., 1988 and EL-Dairouty et al., 1990).

Table 2: Gross composition of blue cheese (style Roquefort) made from goat's milk compared with that o cow's milk

Chemical Composition	Ripening period (days)							
	Goat milk				Cow milk			
	1	30	60		1	30	60	
			A	B			A	B
Moisture	55.70	54.20	52.90	52.70	57.70	56.15	54.50	54.20
Fat	16.20	16.70	17.50	17.55	15.40	16.50	17.10	17.20
Fat/DM	36.57	36.46	37.15	37.10	36.41	37.62	37.15	37.10
Total nitrogen	2.65	2.95	3.20	3.40	2.80	2.95	3.15	3.45
pH value	5.20	5.30	5.40	5.50	5.30	5.50	5.60	5.65

Average of three replicates
A= at room temperature B= at refrigerator temperature

Table (2) represented that total nitrogen of goat's and cow's blue cheese. Total nitrogen content of cow's cheese was similar to goat's cheese at fresh and during ripening, total nitrogen were gradually increased in both cheeses as the ripening progressed, which due to the continuous protein hydrolysis of cheese during ripening. These results were agreed with those of (Abd-EL-Salam et al., 1988, EL-Dairouty et al., 1990 and Farahat et al., 1982).

From Table (2) it can observed that fat% and fat/DM percentage of goat's blue cheese was similar to cow's blue cheese at fresh and during ripening, fat contents were gradually increased in both cheeses as the ripening progressed, which due to the decrease in moisture content of cheese during ripening. These results were agreed with those of (Abd-EL-Salam et al., 1988, EL-Dairouty et al., 1990 and Farahat et al., 1982).

Amino acids content in blue cheese (style Roquefort):

Proteolysis of blue cheese in particularly extensive as a result to the activity of proteinase from the action of exo- and endo peptidases of *Penicillium roqueforti* (Le-Pars and Gryion, 1981). The relative proportions of free amino acids in Blue cheese made from goat's milk compared with that made from cow's milk at fresh and during ripening period, Table (3).

In fresh cheese it can be observed that Aspartic, Glutamic, Proline and Cystine were higher in both cheeses while Aspartic, Glutamine, Glycine, Proline, Leucine and Lysine were higher in cheese made from goat's milk than that made from cow's milk. This trend may be due to the variation of milk protein in both goat's and cow's milk (Juarez and Ramos, 1984). After 30 days of ripening it can be showed that the concentration of Arginine, Valine, Methionine, Cystine, Leucine, Phenylalanine and Lysine amino acids were increased with the same trend in both cheeses, while Therionine amino acid decreased in cow's cheese and increased in goat's cheese. On the other hand amino acid Proline decreased in both cheeses. In the other hand, it observed that Glutamic, Histidine, Therionine and Tyrosine amino acids were decreased in cow's cheese while, goat's cheese were increased except Proline amino acid which decreased. These variations may be

due to the breakdown of protein in Blue cheese (Fernandez-Salguero, et al., 1989).

At the end of ripening (60 days) both cheeses ripened at refrigerator or at room temperature Table

Lysine decreased in goat's cheese and increased in cow's cheese. The remain amino acids increased in goat's cheese, while decreased in cow's cheese during ripening. This decreasing in some amino acids concentration may be attributed to their degradation to another minor components and free fatty acids throughout the decarboxylation and deamination of amino acids (Nakae and Eliott, 1965 and Ali, 1993).

(3), the results evaluated that Aspartic and Glutamic were decreased in both cheeses while, Proline and

Total amino acids in both blue cheese (Style Roquefort) increased during ripening period especially, after 30 days. On the other hand cheese ripened for 60 days at room temperature had a higher total amino acids contents in goat's cheese than that in cow's cheese. Total amino acids were higher when cheese ripened at refrigerator than that ripened at room temperature. These results were agreed with that obtaining by Dolores Gonzales de Liano et al., 1991.

Table 3: Mean values of free amino acids content of Roquefort style cheese made from goat's milk compared with that o cow's milk during ripening.

Amino acids concentration g/100 g cheese protein	Ripening period (days)							
	Goat milk				Cow milk			
	Fresh	30	60		Fresh	30	60	
			A	B			A	B
Aspartic	3.56	1.08	0.12	0.99	1.96	0.98	0.48	1.97
Glutamic	4.37	6.97	0.87	0.02	2.23	1.24	0.95	1.99
Serine	0.19	0.35	0.22	0.22	0.29	0.21	0.09	0.14
Glycine	0.28	0.37	0.19	0.27	0.24	0.23	0.12	0.23
Histidene	0.63	1.30	0.91	0.90	1.29	0.96	0.44	0.53
Argininr	0.64	1.26	0.82	0.72	0.41	0.61	0.30	0.90
Therionine	0.36	3.09	0.60	2.54	1.84	1.62	0.82	0.41
Alanine	0.52	0.59	0.24	0.22	0.38	0.35	0.15	0.28
Proline	3.19	1.72	2.35	1.29	1.39	1.17	0.34	2.76
Tyrosine	0.46	1.19	0.99	1.00	0.72	0.58	0.12	0.88
Valine	0.16	1.61	3.77	1.17	0.99	1.25	0.22	0.51
Methionine	0.01	1.68	0.28	1.47	0.78	1.17	0.13	0.18
Cystine	2.50	27.77	13.44	20.65	4.33	16.51	1.41	0.96
Iso leucine	0.29	0.54	0.63	1.61	0.18	0.37	0.08	0.01
Leucine	0.62	1.58	0.67	1.11	0.20	0.54	0.03	0.10
Phenylalanine	0.66	1.80	0.28	1.68	0.19	0.54	0.20	0.24
Lysine	2.98	3.76	1.16	1.45	0.65	3.08	1.58	0.13
Total	**21.52**	**56.66**	**27.54**	**37.22**	**18.07**	**31.42**	**7.46**	**12.22**

Average of three replicates

A= at room temperature B= at refrigerator temperature

Ripening indices:

Table (4) reflected total volatile fatty acids in both blue cheese (Style Roquefort) increased during ripening period. On the other hand cheese ripened 60 days at room temperature had higher total volatile fatty acids contents in goat's cheese than that in cow's cheese. Total volatile fatty acids content were higher when cheese ripened at room temperature than that ripened at refrigerator. These results were agreed with that obtaining by (Dolores Gonzales de Liano et al., 1991).

Tyrosine and Tryptophan contents were gradually increased in the two treatments with progressive ripening (Table 4). However, at the end of ripening period Tyrosine & Tryptophan contents of goat's cheese were higher than that in cow's cheese. Also, their content was higher of cheese ripened at room temperature than that ripened at refrigerator.

Table 4: Mean values of total volatile fatty acids, Tyrosine and Tryptophan blue cheese made from goat's milk compared with that of cow's milk during ripening.

Ripening Period	TVFA		Tyrosine		Tryptophan	
	Cow	Goat	Cow	Goat	Cow	Goat
1	3.8	3.4	10.6	13.8	19.8	23.8
30	10.6	12.6	29.9	38.6	24.6	36.5
60 room temperature	31.2	35.2	88.3	110.3	56.5	60.9
60 refrigerator	22.8	20.0	54.1	63.2	36.8	43.3

Average of three replicates

Table 5: The sensory evaluation of blue cheese made from goat's milk compared with that of cow's milk during ripening.

	One month		Two months			
			Room temperature		Refrigerator	
	Cow	Goat	Cow	Goat	Cow	Goat
Colour (40)	22	24	36	38	26	28
Flavour (40)	32	36	32	38	34	36
Texture(20)	16	16	16	18	18	18
Total (100)	70	76	84	94	78	82

Average of three replicates

Organoleptic properties:

Table (5), shows the sensory evaluation of blue cheese (Style Roquefort) during ripening. Blue goat's cheese was ranked the highest total score (76/100) when one month old. Also, the same cheese ranked the highest total score (94/100) at the end of ripening period (60 days) when cheeses were ripened at room temperature. Blue cheese made from goat's was ranked higher flavour score than that made from cow's milk during ripening period. The cheese ripened at room temperature had higher total score than that ripened at refrigerator.

4. Conclusion

It can be concluded that blue cheese (Style Roquefort) can be successfully made from goat's milk, the resultant cheese had a similar properties compared with that made from cow's milk. Also, this cheese had acceptability over than that made from cow's cheese and was also, ranked the highest total score during ripening.

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Manufacture of Cultured Butter Milk Beverage from Whole and Skimmed Goat's Milk

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Abstract: The development of high quality cultured butter milk beverage (CBMB) is primarily dependent on a controlled fermentation of the milk constituents. Cultured butter milk beverage was made from either whole or skim goat's milk, using mesophilic L-starters FR 19-8126 (Lacfococcus lactis subsp.lactis, Lact. cermohs subsp.cremoris and Leuconostoc cremohs) and DL-starters A-8101 (the same of microorganisms L-starters contain plus Lact. lactis Subs, diacetilactis). Chemical, flavour and organoleptic properties of the resultant four CBMB treatments were compared, when fresh and during 15 days of storage at 7°C. The CBMB made from goat's whole milk cultured with DL-starters had diacetyl and acetaldehyde values which were reported to be necessary for a good flavour balance. Moreover, it received the highest organoleptic scores. Therefore, this CBMB was recommended to be produced commercially in Egypt.

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Keywords: Cultured butter milk, Goat's milk, L- starter, DL-starter.

1. Introduction

Goat's milk is a very good source of calcium and the amino acid tryptophan. It is also a good source of protein, phosphorus, riboflavin (vitamin B2) and potassium. Perhaps the greatest benefit of goat's milk, however, is that some people who cannot tolerate cow's milk are able to drink goat's milk without any problems. It is not clear from scientific research studies exactly why some people can better tolerate goat's milk. Some initial studies suggested that specific proteins known to cause allergic reactions may have been present in cow's milk in significant quantities yet largely absent in goat's milk, Cheng, et. al.(2005); Elwood, et al. (2007); Ensminger and Esminger (1986).

Goat's milk has a more easily digestible fat and protein content than cow milk. The increased digestibility of protein is of importance to infant diets. Goat milk can successfully replace cow milk in diets of those who are allergic to cow milk. In under-developed countries, where meat consumption is low, goat milk is an important daily food source of protein, phosphate and calcium (Paul kindstedt, 2005).

The origin of fermented milks in the diets of humans date back many thousands of years and predates the existence of written records of their production and consumption. Fermented milks were produced since 10,000-15,000 years ago as man's way of life changed from being food gatherer and hunter to food producer. It is likely that this transition may have occurred at different times in different parts of the world. However, archaeological evidence shows some civilizations e.g. the Sumerians and Babylonians in Mesopotamia, the Pharoes of ancient Egypt and the Indians in Asia were well advanced in agricultural and animal husbandry y methods and kept cows and buffalos for milk production, which was either consumed as such or processed into other products (Bill,2009).

Originally, butter milk is a by-product of butter making, now became a milk product cultured with lactic acid. It is available in both whole and skim milk, the cultured butter milk beverage (CBMB) is a fluid with suitable viscosity, of typical clean refreshing acid taste with pleasant flavour and aroma (Alm, 1982a). It is usually consumed fresh, and should be kept refrigerated throughout distribution. CBMB is usually manufactured from pasteurized milk (Whole or skim), using mesophilic mixed-strain starter cultures containing acid producing Lactococcus lactis subsp.lactis and Lact. cremoris subsp.cremoris, and flavour producing either Leuconostocs (L-starter) or both the Leuconostocs and Lact. diacetilactis (DL-starter).

Relatively, little information exists about the effect of goat's milk and skimming of milk and starter type on the chemical, flavour and organoleptic properties of CBMB. So, this was the object of the present paper.

2. Materials and Methods

Fresh bulk goat's milk (whole milk) was obtained from a private sector at Dakahliah Governorate, Egypt. It had an average composition of 13.14% T.S., 4.2% fat, 3.87% lactose, 0.16% acidity, 4.43% protein, and pH 6.63. Skim milk was obtained from the whole milk using a mechanical separator, it

contained 9.43 S.N.F. Mesophilic L-starters (FR-198126) and DL-starters (A-8101) were obtained from the Netherlands Institute for Dairy Research (Nizo, Ede, Netherlands). They are usually used for the production of Dutch-cultured butter milk and other cultured dairy products (Noomen et al, 1992). The L-starters contained Lacfococcus lactis subsp. lactis, Lact. cermoris subsp. cremoris and Leuconostoc cremoris, were used to inoculate to CBMB. Addition to these microorganisms the DL-starters contain Lact. lactis Subs, diacetilactis were used to inoculate the other treatment.

Cultured butter milk beverage treatments were made from:

Tr.1. Whole milk cultured with L-starters.
Tr.2. Whole milk cultured with DL-starters.
Tr.3. Skim milk cultured with L-starters.
Tr.4. Skim milk cultured with DL-starters.

Manufacture of cultured butter milk beverage:

Manufacturing was done according to the method of Walker and Gilliland (1987).

Then cooled to 7°C, bottled and stored in a refrigerator. Samples were taken from the CBMB after fermentation and after storage for 3, 5, 7 and 15 days at 7°C. Triplicate samples were taken for analysis.

Chemical analysis:

The pH was measured using a digital pH meter with combined electrode, total solids (T.S), fat, acidity, lactose, total protein and non-protein nitrogen (NPN) percentages were determined according to AOAC (2000). The total volatile fatty acids (TVFA) were estimated according to Kosikowski (1984); diacetyl according to Less & Jago (1970), and acetaldehyde by using method of Lees and Jago (1969). The CBMB from different treatments was scored for organoleptic properties by a panel 20 staff members of Food Technology and Dairying Department, National Research Centre. The assessed properties were: flavour (40), acidity (20), colour (10) and consistency (30).

3. Results and Discussion

Chemical composition

The total solids, fat and protein contents of CBMB from whole goat's milk, and skim milk were not significantly affected either by starter type or storage period (tables1,2). This could be explained on the basis that mesophilic lactis bacteria has low lipolytic and proteolyic activity towards milk fat and protein, respectively, (Alm,1982b,d) and Law & Kolstada (1983). However, the NPN content of CBMB slightly increased after fermentation and during storage (Tables 1, 2). This could be explained on the basis that the added starter may cause a limited hydrolysis of some whey proteins (Alm, 1982d). However, the NPN content of CBMB was not significantly affected by milk skimming or starter type.

The amount of lactose of CBMB generally, decreased after fermentation (at fresh) in all treatments (Tables 1, 2) ranging from 3.87 to 3.71%. This range was in accordance with those reported for Swidish-Fresh CBM (Alm, 1982b). The lactose content proportionally decreased with increasing keeping time of CBMB. At the end of storage time (15 days), the amounts of lactose were in the range of 2.90-2.50% in all samples, indicating that the fermentation of lactose was continued but at a relatively low rate during storage. The changes in the pH values of CBMB from different treatments coincided with the decrease in lactose content with increase acidity in all treatments. They decreased significantly after fermentation. At this pH range, most bacterial growth could be inhibited, which makes the fermented product biologically safe (Walestra et al., 1993). During storage the pH value of CBMB with the use of DL-starier slightly decreased than with the use of L-starter. Also, pH of CBMB from skim milk was generally less than that from whole milk.

Table (1): Chemical composition of cultured butter milk beverage (CBMB) from whole goat's milk during storage.

	Tr.1						Tr.2					
days	0	3	5	7	10	15	0	3	5	7	10	15
TS%	13.2	13.2	13.3	13.3	13.4	13.5	13.1	13.1	13.2	13.3	13.3	13.42
Fat%	4.2	4.1	4.1	3.9	3.5	3.5	4.4	4.4	4.3	4.2	4.0	3.6
Protein %	4.43	4.43	4.50	4.52	4.58	4.71	4.16	4.18	4.26	4.41	.48	4.52
NPN%	0.03	0.03	0.03	0.04	0.05	0.05	0.03	0.03	0.04	0.04	0.04	0.045
Lactose%	3.71	3.60	3.41	3.18	2.86	2.70	3.87	3.63	3.48	3.13	3.01	2.90
pH	5.0	4.84	4.71	4.51	4.50	4.46	5.2	5.0	4.94	4.88	4.81	4.76
Acidity%	0.95	1.01	1.09	1.18	1.21	1.26	0.92	0.97	1.02	1.03	1.17	1.19

T1- Whole milk + L-starters. T2- Whole milk + DL-starters

Table (2): Chemical composition of cultured butter milk beverage (CBMB) from skim goat's milk during storage.

	Tr.3						Tr.4					
days	0	3	5	7	10	15	0	3	5	7	10	15
TS%	9.43	9.43	9.43	9.49	9.53	9.58	9.24	9.24	9.28	9.31	9.34	9.38
Protein %	4.18	4.18	4.22	4.26	4.30	4.32	4.43	4.43	4.45	4.49	4.51	4.58
NPN%	0.03	0.033	0.04	0.04	0.05	0.059	0.036	0.04	0.053	0.058	0.062	0.068
Lactose%	3.71	3.60	3.76	3.08	2.66	2.50	3.77	3.36	3.42	3.08	2.91	2.72
pH	4.4	4.3	4.2	3.9	3.81	3.62	4.32	4.26	4.20	3.86	3.65	3.57
Acidity%	1.24	1.28	1.31	1.34	1.40	1.44	1.13	1.13	1.19	1.22	1.28	1.33

T3- Skim milk + L-starters. T4- Skim miik + DL-starters.

The development of acidity are presented in Fig. 1. They generally supported the results of lactose and pH determinations. The acids consisted mainly of lactic acid and a little of acetic acid (Walestra et.al, 1993). In figure 1, the acidity of CBMB increased during storage, these results were generally in accordance with other reported by Alm, 1982c; Noomen et al., 1992 and Varnam & Sutherland, 1994 . During storage, the acidity gradually increased to reached to 1.44% after 15 days of storage. Kosikowski (1984), reported similar increase in acidity during storage of CBMB. The results also indicated that the use of skimmmilk, with L-starter gave more acids in CBMB than whole milk and DL-starters, respectively. Using standardized cow's milk with less than 0.4% fat developed excess acid flavour in Dutch CBM (Noomen etal., 1992).

Total volatile fatty acids (TVFA)

The TVFA values (Fig. 2) were increasing during storage. These results were in accordance with those reported by (Alm, 1982 a). At the end of storage time (15 days), the TVFA were relatively high in CBMB made with the use of DL-starters compared to the CBMB made with L-starters.

Diacetyl and Acetaldehyde

Fig: 3 and 4 shows that DL-starters produced more diacetyl and acetaidehyde after fermentation than L-starters in CBM made from the same milk. This could be explained on the basis that DL-starters have the ability to ferment citric acid more rapidly and produce more diacetyl than L-starters (Walstra et al., 1993). During 15 days of storage, the diacetyl content gradually increased indicating that the flavour-producing strains (Leuconostocs and or Lactococcus diacetilactis) in the added starters remained active during this periods. On the other hand, the acetaidehyde content proportionally decreased during storage. The loss of acetaidehyde occurs during storage might be due to the ability of Leuconostocs to convert part of the acetaidehyde forming ethanol which was reported to have no role in the flavour of CBM (Collins & Speckman, 1972 and Varnam & Sutherland, 1994). Since the diacetyl : acetaidehyde ratio is near the 4:1, which is considered to be necessary for a good flavour balance in CBM (Lindsay et. al., 1965 and Badings, 1984). This desired ratio was hard to be found in the commercial buttermilk sample analyzed by Keenan et al. 1968 and Vasavada & White, 1979.

Organoleptic properties

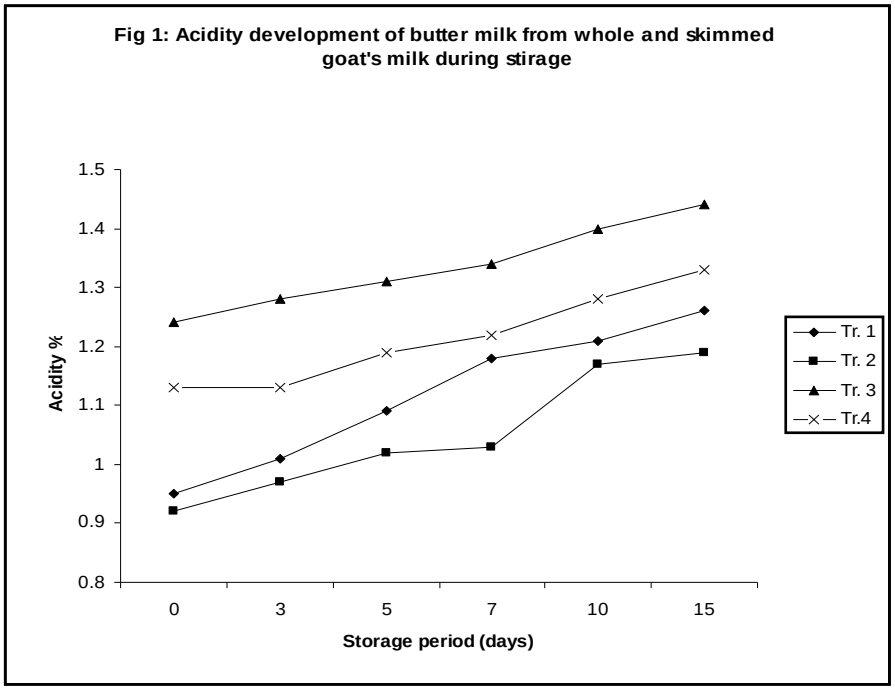
Table (3) shows organoleptic scores of the CBMB treatments when fresh and after storage. All CBMB treatments appeared like fluid without wheying-off and gained high scores for consistency and colour. Fluidity is reported to be ideal for CBM, it also increases its refreshing quality (Kosikowki, 1984). After fermentation, the scores of consistency and colour showed no differences due to the different starters used, while they showed slightly lower values when using skim milk than whole milk. At the end of storage time, all the samples were scored lower. Furthermore, all the samples had clean and refreshing acid taste with pleasant flavour and aroma. They generally have good scores for acid and flavour. CBMB made from whole milk cultured with L-starters showed higher score than the other samples. The total organoleptic score, however, gradually decreased with advancing storage time.

4. Conclusion

In conclusion, the manufacture of CBMB from whole goat's milk using DL-starters can be recommended as it had well balanced flavour and good organoleptic properties.

Table (3): Organoleptic properties of cultured buttermilk beverage from goat's milk during storage.

Treatment	Storage period (Days)	Organoleptic properties score				
		Flavour 40	Acid 20	Colour 10	Consistency 30	Total 100
Tr. 1 Whole milk + L-starter	0	35	16	9	29	89
	3	32	16	9	29	86
	5	32	16	9	29	86
	7	29	16	8	24	77
	10	27	14	8	24	73
	15	24	14	8	23	69
Tr. 2 Whole milk + DL-starter	0	36	16	9	27	88
	3	33	16	9	27	85
	5	33	14	9	27	83
	7	29	14	8	23	74
	10	27	14	8	23	72
	15	20	12	8	18	58
Tr. 3 Skim milk + L-starter	0	38	16	8	20	82
	3	38	16	8	20	82
	5	34	16	7	20	77
	7	29	14	7	17	67
	10	29	14	7	17	67
	15	27	12	5	15	59
Tr. 4 Skim milk +DL-starter	0	38	18	8	20	84
	3	38	18	8	20	84
	5	34	18	7	20	79
	7	29	16	7	17	69
	10	29	16	7	17	69
	15	27	14	5	15	61



Tr.1. Whole milk cultured with L-starters. Tr.2. Whole milk cultured with DL-starters.
T3. Skim milk cultured with L-starters. Tr.4. Skim milk cultured with DL-starters

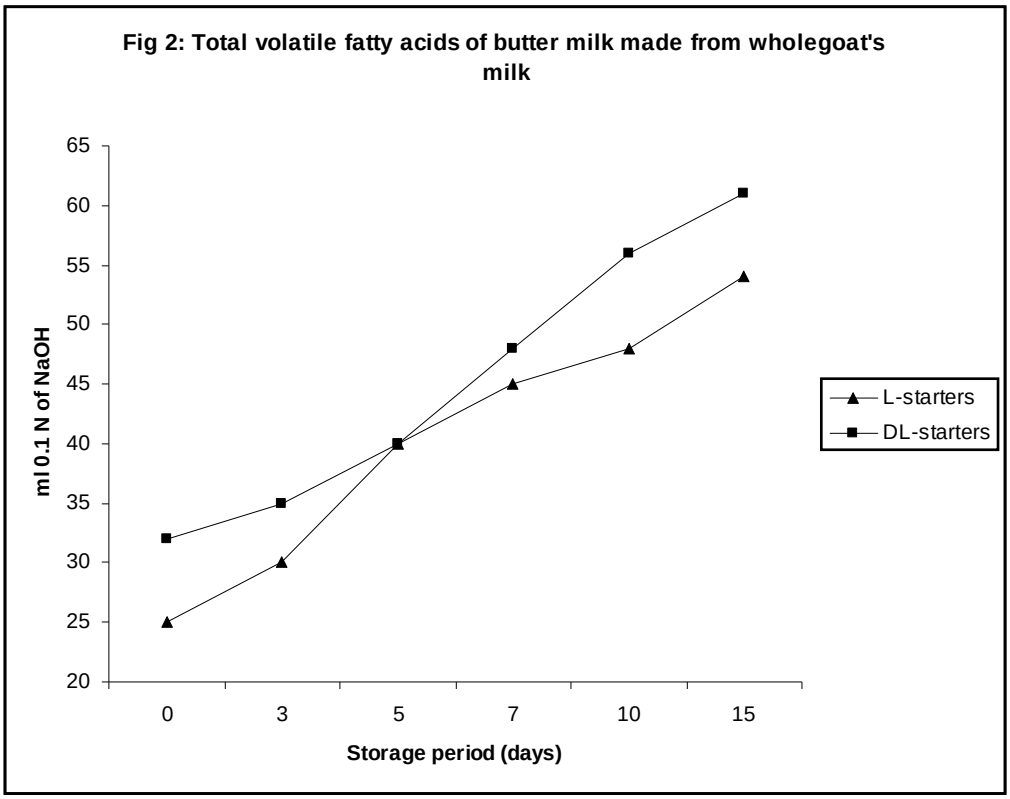


Fig 2: Total volatile fatty acids of butter milk made from wholegoat's milk
ml 0.1 N of NaOH
65
60
55
50
45
40
35
30
25
20
0
3
5
7
10
15
Storage period (days)
L-starters
DL-starters

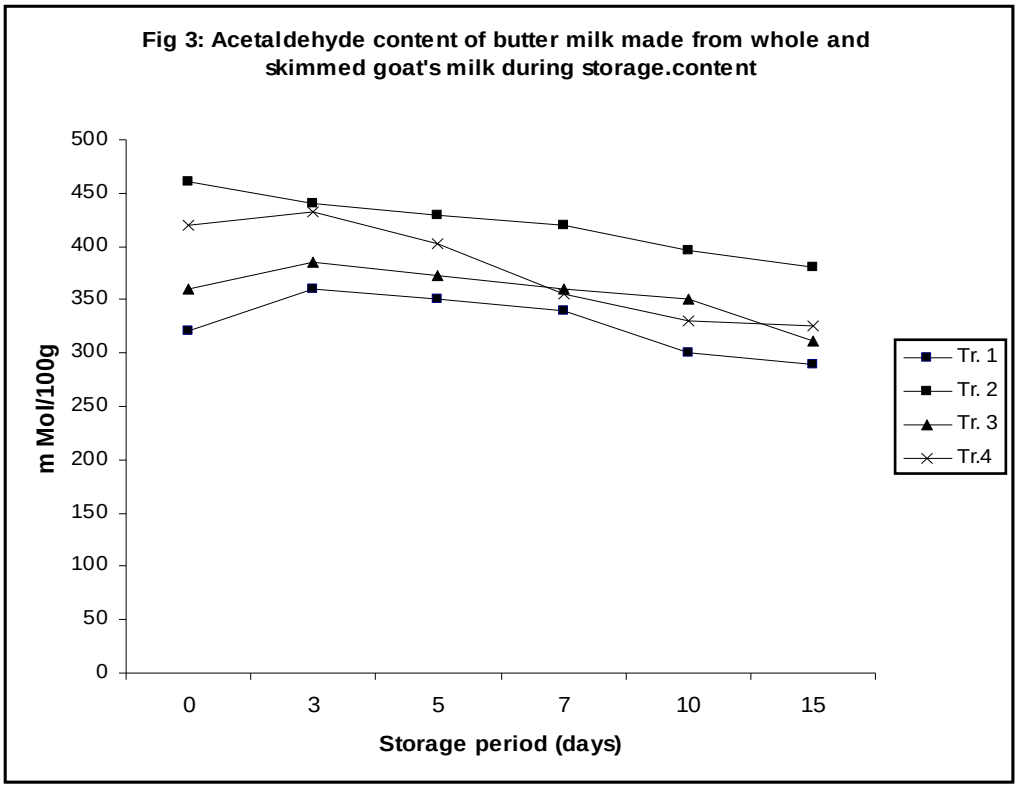
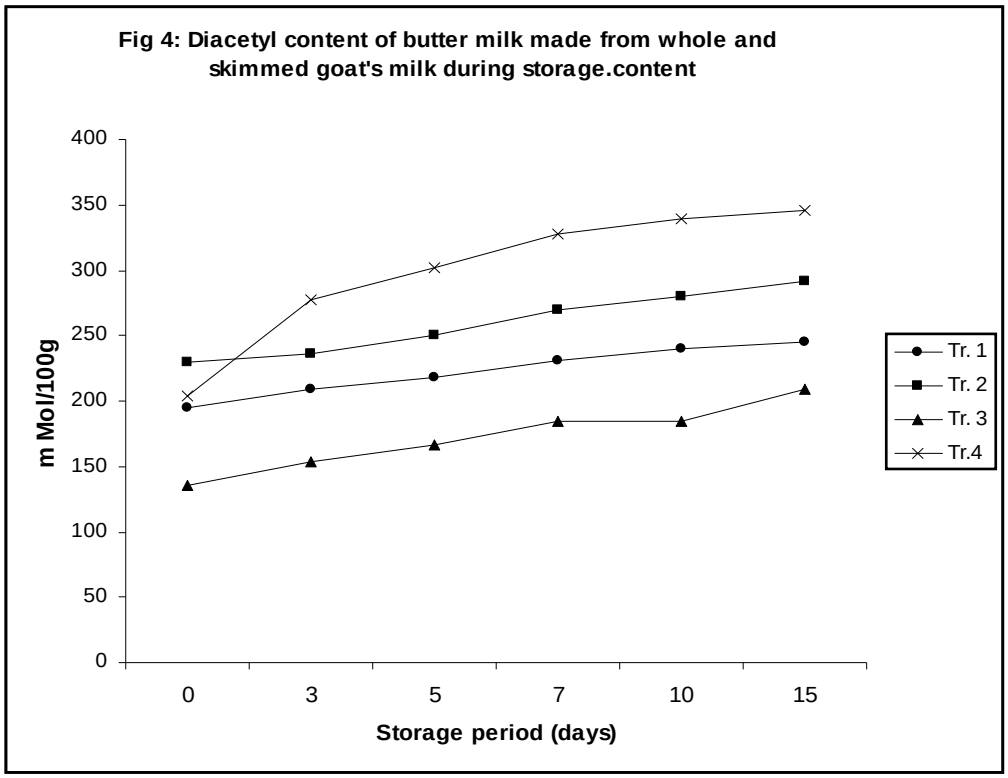


Fig 3: Acetaldehyde content of butter milk made from whole and skimmed goat's milk during storage.content
m Mol/100g
500
450
400
350
300
250
200
150
100
50
0
0
3
5
7
10
15
Storage period (days)
Tr. 1
Tr. 2
Tr. 3
Tr.4



Tr.1. Whole milk cultured with L-starters. Tr.2. Whole milk cultured with DL-starters.
Tr.3. Skim milk cultured with L-starters. Tr.4. Skim milk cultured with DL- starters

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Well Logs Application in Determining the Impact of Mineral Types and Proportions on the Reservoir Performance of Bahariya Formation of Bassel-1x Well, Western Desert, Egypt.

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Abstract: The present work dealt with the computerized well log analysis of Bassel – 1X well in the Sherouk Field in the Northern Western Desert of Egypt to determine the mineralogical composition of Lower and Upper Bahariya Formation and to estimate the influence of these minerals on the different petrophysical parameters of Lower and Upper Bahariya Formation. The lithologic and mineralogical compositions were identified qualitatively through the utilizing of crossplots which were established by using the different petrophysical parameters. Also the lithologic and mineralogical compositions were established quantitatively by using the mathematic equations. The matrix components of Lower Bahariya Formation included few percentage of clay minerals (illite and montmorillonite) and high quantity of quartz, calcite and dolomite, while in Upper Bahariya Formation it involves high percentage of clay minerals (illite and montmorillonite) and low quantity of quartz, calcite and dolomite. These minerals were plotted against the different petrophysical parameters to show the effect of these minerals on the effective porosity and the saturation of hydrocarbon.

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Keywords: Logs; Mineral Type; Reservoir; Bahariya; Western Desert; Egypt

Introduction:

The way of analysis of open – hole well log data is an important technique for studying the rock characteristics such as matrix identification, from which the mineralogical composition can be detected (Ramadan, 1997). The mixed lithology possesses a particular problem for the log analyst because the difficulties arise from the mineralogical complexity reflected from varying rock associations, facies and depositional environments. By this way, the demand for appropriate method and techniques to evolve the lithologic problem is advisable (Said et al. 2003). So the crossplots are important for establishing the lithology and mineralogy for the rock units qualitatively.

First the lithology is identified through M – N and ρ_b - Φ_N plots and then the minerals are defined through Th – K, M lith and mid plots. Secondly, the mineral compositions are established by using the mathematical modeling (Mats, et al. 2004).

The different petrophysical parameters such as the effective porosity and the hydrocarbon saturation (movable and residual) are greatly affected by the presence of clay minerals. In this study the mineral composition will be evaluated and will show its effect on the petrophysical characteristic for Lower and Upper Bahariya Formation in Bassel – 1x well. This well is located at latitude 30° 35\ 10.1\\ N and Longitude 26° 56\ 36.9\\ E in the Sherouk Field in the northern western Desert of Egypt (Fig. 1).

Crossplots Application:

The well log data extracted for the studied rock units are presented in the form of crossplots which, assist in the selection of the interpretation parameters and the identification of the trend and problems of the mineralogical models.

On all types of crossplots, it is of prime importance to locate the position of the most probable presented minerals. The following is a detailed presentation for the various crossplots constructed for the evaluated rock units (Lower and Upper Bahariya Formation) in the studied well.

1- ρ_b vs.Φ_N – GR. Z plot and M – N plot:

These plots (Figs. 2 and 3) represent the relation between ρ_b and Φ_N with taking the GR as a third component.

The first glance reveals the chemical nature of these rock units. The majority of the presented points in this plot lie between the Limestone, Dolomite and the sand line with high and medium GR intensity for the Upper Bahariya Formation, indicating the presence of shale but Low GR intensity in the Lower Bahariya Formation. Some points are located around illite, kaolinite and montmorillonite.

Figures (4 and 5) show nearly the same lithologic composition as in the previous figures, the calculation of M and N will be shown in M-N lith plot.

2- Mid plot:

It is a relation between the apparent matrix parameters $(\rho_{ma})_a$ and $(\Delta t_{ma})_a$, which are computed (Wyllie, 1963) as the following:

a) For clean zones:

$\rho_{b\ log} = \Phi\rho_f + (1 - \Phi)\ \rho_{ma}$ or $\rho_{ma} = (\rho_{b\ log} - \Phi\rho_f) / (1 - \Phi)$…..(1)

$\Delta t_{log} = \Phi\Delta t_f + (1 - \Phi)\ \Delta t_{ma}$ or $\Delta t_{ma} = (\Delta t_{log} - \Phi\Delta t_f) / (1 - \Phi)$….. (2)

b) For shaly zones:

$\rho_{b\ log} = \Phi\rho_f + V_{sh}\ \rho_{sh} + (1 - \Phi - V_{sh})\rho_{ma}$ or

$\rho_{ma} = (\rho_{b\ log} - \Phi\rho_f - V_{sh}\ \rho_{sh}) / (1 - \Phi - V_{sh})$…..(3)

$\Delta t_{log} = \Phi\Delta t_f + V_{sh}\ \Delta t_{sh} + (1 - \Phi - V_{sh})\ \Delta t_{ma}$ or

$\Delta t_{ma} = (\Delta t_{log} - \Phi\Delta t_f - V_{sh}\Delta t_{sh}) / (1 - \Phi - V_{sh})$….. (4)

Clavier and Rust (1976) proposed this crossplot which shows the separation of the different matrix contents. Figures (6 and 7) are plots for Lower and Upper Bahariya Formation in the studied well. In the Lower Bahariya Formation, Figure (6) shows a group of points lying between the Quartz, K-feldspars and carbonate in the form of calcite and dolomite, while the plot of the Upper Bahariya Formation (Fig. 7) exhibits that the majority of the points around the clay minerals (illite and montmorillonite).

3- M lith – N lith crossplot:

The advantage of is this plot that, it depends on the three porosity logs (ρ_b, Δt, and Φ_N), from these values, two parameters (M and N) are calculated (Burke et al. 1969). These parameters are calculated by the following formulae:

M lith = $((\Delta t_f - \Delta t_{log}) / (\rho_{b\ log} - \rho_f)) * 0.01$….. (5)

N lith = $(\Phi_{Nf} - \Phi_{N\ log}) / (\rho_{b\ log} - \rho_f)$….. (6)

Figure (8) reflects that calcite; Quartz and K-feldspars are presented in this crossplot while the clay minerals (montmorillonite, kaolinite) are shown in the crossplot of Upper Bahariya Formation (Fig. 9).

4- Th – K crossplot:

It is one of the best crossplots which identify the presence of clay minerals where, the NGS (U, Th and K content) tool is the best logs for discriminating the clay minerals.

The analyzed crossplots (Figs. 10 and 11) reveal the presence of some illite in the Lower Bahariya Formation while, existence of mixed layer clay, illite and montmorillonite can be observed in the Upper Bahariya Formation.

Mathematical Modeling:

Based on the mineralogical modeling obtained from the previous technique, the mathematical modeling was established. Through this modeling process, one has to select the equations, which will enable to relate the log data to the desired computed parameters, like mineral constituents and porosity (Abu El-Ata et al., 1985). The response equations of the minerals, present in each model, are performed and a statistical analysis is carried out on each mineral, in a probabilistic test, for establishing the mineralogical composition that frequently occurred in each studied zone (Delfiner et al., 1984).

Based on the mineralogical model of the Lower and Upper Bahariya Formation in Bassel -1x well, the two units are composed of Qz, calcite, dolomite, illite and montmorillonite with different percentages. The following equations (7-10) are used for Lower Bahariya Formation while, the equations (11-14) represent the Upper Bahariya Formation.

ρ_b=2.65qz+2.71cal+2.88dol+2.52ill+2.12 mont+1.0Φ…..(7)

Φ_N=-0.02qz–0.01cal+0.01dol+0.30ill+0.44 mont+1.0Φ….. (8)

ΔT=55.5qz+48cal+43.5dol+120ill+140mont+189Φ…. (9)

1=Vqz+Vcal+Vdol+Vill+Vmont+Φ….. (10)

ρ_b=2.65qz+2.71cal+2.88dol+2.52ill+2.12 mont+1.0Φ…..(11)

Φ_N=-0.02qz–0.01cal+0.01dol+0.30ill+0.44 mont+1.0Φ….. (12)

ΔT=55.5qz+48cal+43.5dol+120ill+140mont+189Φ…. (13)

1=Vqz+Vcal+Vdol+Vill+Vmont+Φ….. (14)

Where:

ρ_b is the reading of density log.

Δt is the interval transit time in μsec/feet.

V is the volume of mineral to be computed (fraction).

Vqz, Vcal, Vdol, Vill and Vmont are the volumes of quartz, calcite, dolomite, illite and montmorillonite

Φ is the porosity value in fraction.

Accordingly, the previous concluded mineralogical models concerning the types of mineral constituents and their volumes, as well as the pore volumes could be helpful in the final lithologic-geologic modeling of the evaluated rock units in the studied well.

Minerologic Quantification:

The determination and calculation of the mineralogical composition and porosity of Bahariya Formation in the studied well is done by using the simultaneous equations as follow;

$$a_1 V_1 + a_2 V_2 + a_3 V_3 = \Phi_D$$
$$b_1 V_1 + b_2 V_2 + b_3 V_3 = \Phi_N$$
$$V_1 + V_2 + V_3 = 1.0 \quad (15)$$

Where : a_1, a_2 and a_3 are the density log readings and b_1, b_2 and b_3 are the neutron log porosity readings of the three rock components; in which their volumes are V_1, V_2 and V_3.

Equation (15) is known as the identity equation and it defines the fact that, these components add up to 1.0. The first component could be the porosity and the other two could be illite and calcite or quartz and montmorillonite (Abu El-Ata and Ismail, 1985).

In the matrix form, these equations should be reduced to:

$$\begin{bmatrix} V_1 \\ V_2 \\ V_3 \end{bmatrix} \begin{bmatrix} a_1 & a_2 & a_3 \\ b_1 & b_2 & b_3 \\ 1.0 & 1.0 & 1.0 \end{bmatrix} = \begin{bmatrix} \varphi_D \\ \varphi_N \\ 1.0 \end{bmatrix} \quad (16)$$

To solve this matrix for the values of V_1, V_2 and V_3, the matrix inverse should be obtained (Delfiner et al., 1984) as;

$$\begin{bmatrix} V_1 \\ V_2 \\ V_3 \end{bmatrix} = \begin{bmatrix} x_1 & x_2 & x_3 \\ y_1 & y_2 & y_3 \\ z_1 & z_2 & z_3 \end{bmatrix} \times \begin{bmatrix} \varphi_D \\ \varphi_N \\ 1.0 \end{bmatrix} \quad (17)$$

This concept can be extended to as many equations as; there are independent log readings available for a well. For example, a full suite of logs may consist of the following:

Spectral Gamma-Ray Log: SGR, uranium, thorium and potassium. Usual Porosity Logs: density, neutron and sonic. Litho-Density Logs: PEF. Usual Resistivity Logs: shallow, medium and deep. Electro-Magnetic Propagation Time Log: EPT.

These logs together with the identity equation give a possibility of 13 equations in 13 unknowns. It is unlikely that, the three resistivity logs are truly dependent equations, so they may not assist in the solution.

The possibility of a ridiculous solution can be reduced by taking all combinations, but five or six equations from the possible unknowns are better for checking the results of each solution for reasonableness. Reasonableness might be performed by reaching a solution, in which the deduced volumes are all within the range of –0.05 to 1.05 (i.e., no material can be presented in large negative quantities or be greater than the volume of rock). However, the method for selecting the desired solution from the various possible ones is to scan the rock types found in all reasonable solutions and to pick a set of rocks that occur most frequently (Szendro, 1983). Moreover, if more log types (known data) are available than the unknowns (mineral volumes), the case is called over - determined. In this situation, the number of equations provided by the logs exceeds the number of components.

An appropriate estimate for the zone composition can be drawn from a least-squares model, in which the error is minimized between the log responses and their corresponding values predicted by the solution, thus the matrix solution of the least-squares model becomes:

$$V = (C^T C)^{-1} C^T L \ldots\ldots (18)$$

where each letter signifies an array of numbers or unknowns, rather than a single number of unknowns, as in the conventional algebra. The "known" are C (vector of log readings), L (vector of log response), the symbol C^T signifies as the transpose of the C matrix, which simply means a matrix in which the rows and columns have been interchanged and the "unknown" is V (vector of the volume of minerals), as shown by Doveton (1996).

On the other hand, if the log types (known data) are less than the unknowns (mineral volumes), the case is called underdetermined, and then the matrix solution for the least-squares model becomes:

$$V = CT (C C^T)^{-1} L \ldots\ldots (19)$$

The different computer programs were established by Reda (1997) for facilitating the complexities arisen in the solution of the simultaneous equations in this study. By this way, the correct values of the mineralogical constituents (quartz, calcite, dolomite, illite and montmorillonite) were derived for Bahariya Formation in the studied well.

Impact of Mineral Types and Proportions on the Reservoir Performance:

As long as production is concerned for any study, the well log data analysis provides a continuous record for the petrophysical parameters. The reservoir porosity (Φ), permeability, water saturation (Sw) and hydrocarbon saturation (Sh) are the most important properties that define and control qualitatively and quantitatively the reservoir performance. The minerals present in the reservoir especially the clay mineral (Moll, 2001) can play the utmost role, which affect both the reservoir capacity and production because the grain size of clay minerals is generally very small and result in very low effective porosity and permeability, thus any presence of clay in a reservoir may have direct consequences on the reservoir properties (Said et al. 2003). However the type of clay minerals must be taken into account in reservoir evaluation. Their influence has been studied by Nesham (1977) which cause pore filling, pore lining or pore bridging. The following discussion represents an attempt to relate the mineralogic suit for each formation to reservoir characteristics.

The lithologic and clay minerals identification of the Upper Bahariya Formation in Bassel – 1x well reveals the majority of shale followed by sandstone and limestone in decreasing occurrence. The clay minerals are represented by illite and

montmorillonite. The calculated reservoir parameters responding for these mineral contents are shown in Fig. (12), it reveals that the high percentage of illite and montmorillonite affected on the reservoir performance of Upper Bahariya Formation, these fine grains of clay minerals reduced the total porosity into small effective porosity this gives rise to varying total hydrocarbon saturation which behaves by analogous way, in which the movable hydrocarbon saturation became the least and residual hydrocarbon saturation became the largest. This reflects badly fair reservoir performance for the Upper Bahariya Formation.

The mineral contents of Lower Bahariya Formation (qz, calcite, dolomite, illite and montmorillonite) play another role for the reservoir performance (fig. 13), Mmontmorillonite and illite decrease in percentage in the Lower Bahariya Formation as compared to the Upper Bahariya Formation which increases the effective porosity and then the hydrocarbon saturation. Moreover, a part of the calcite is dolomitized, giving rise to more secondary porosity. These variations of the mineral types and their contents enhanced the reservoir performance of Lower Bahariya Formation as compared to that of the Upper Bahariya Formation.

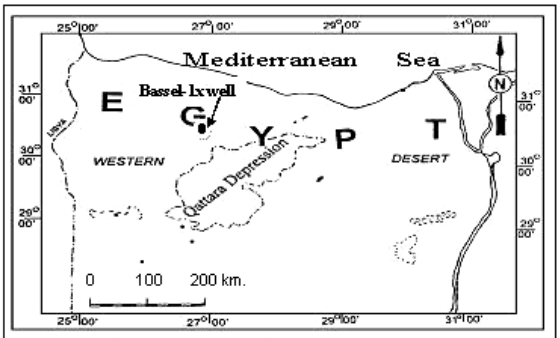

Fig.(1) Location Map of the Studied Well in the Western Desert

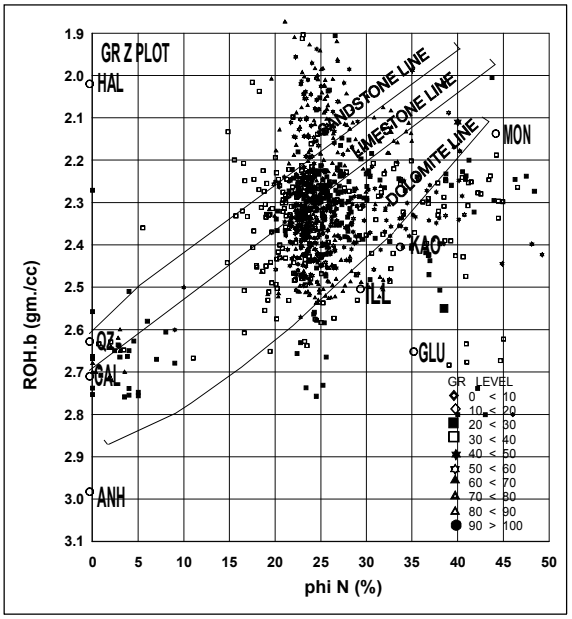

Fig. (2) Density-Phi Neutron with SGR Z-plot of Lower Bahariya Formation for Bassel-1x well.

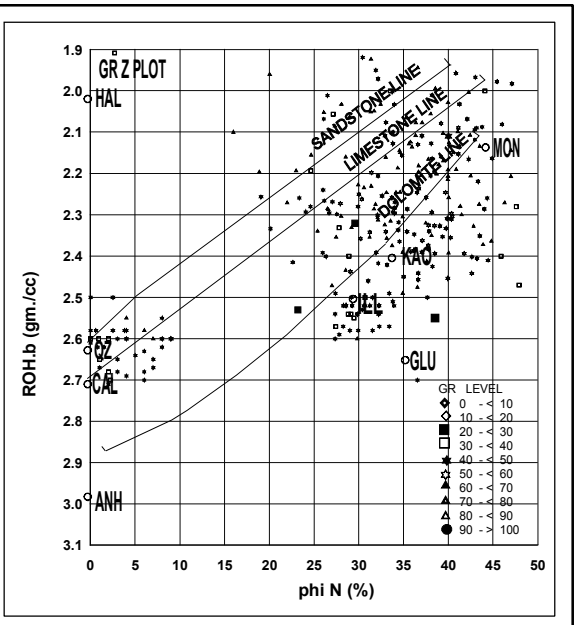

Fig. (3) Density-Phi Neutron with SGR Z-plot of Upper Bahariya Formation for Bassel-1x well.

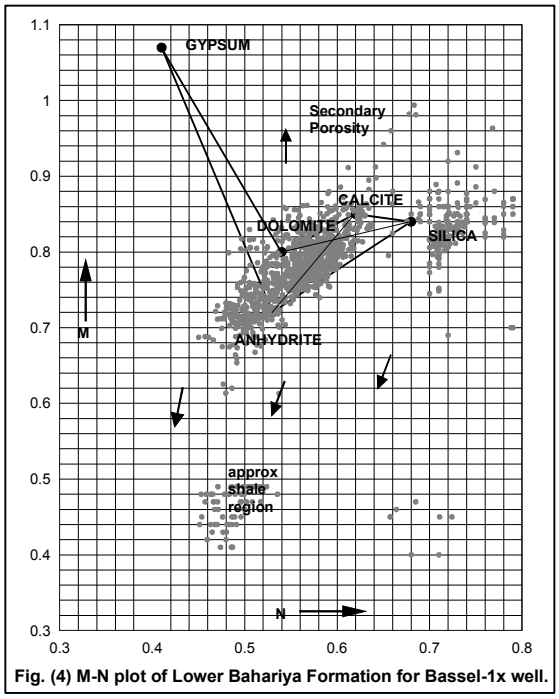

Fig. (4) M-N plot of Lower Bahariya Formation for Bassel-1x well.

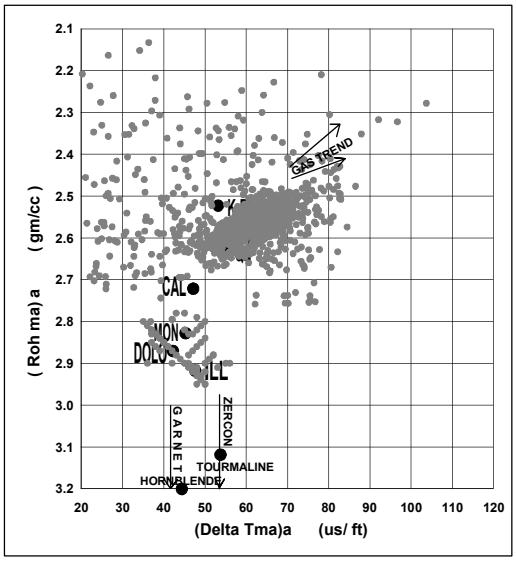

Fig. (6) Mid plot of Lower Bahariya Formation for Bassel-1x well.

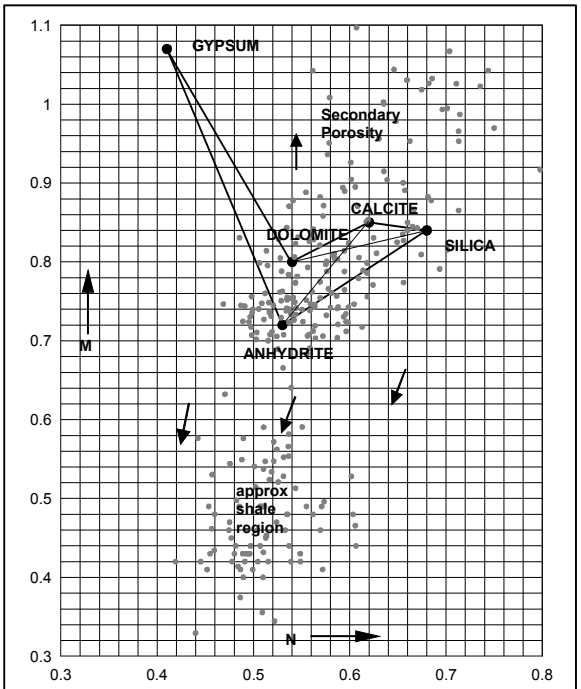

Fig. (5) M-N plot of Upper Bahariya Formation for Bassel-1x well.

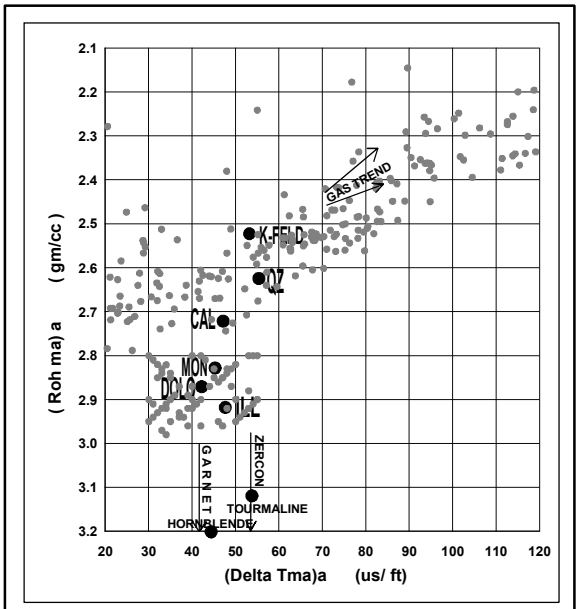

Fig. (7) Mid plot of Upper Bahariya Formation for Bassel-1x well.

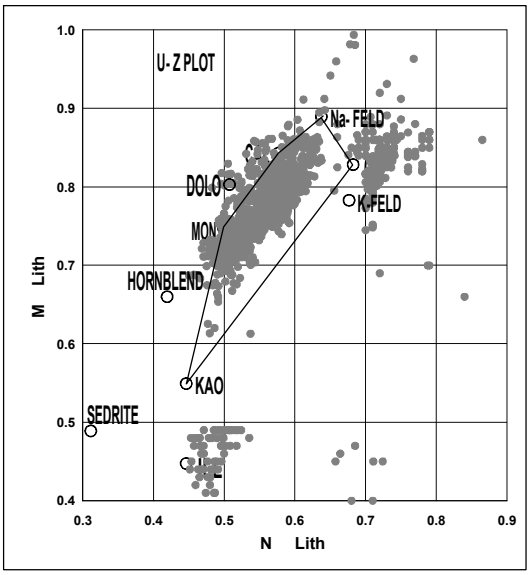

Fig. (8) M lith-N lith crossplot of Lower Bahariya Formation for Bassel-1x well.

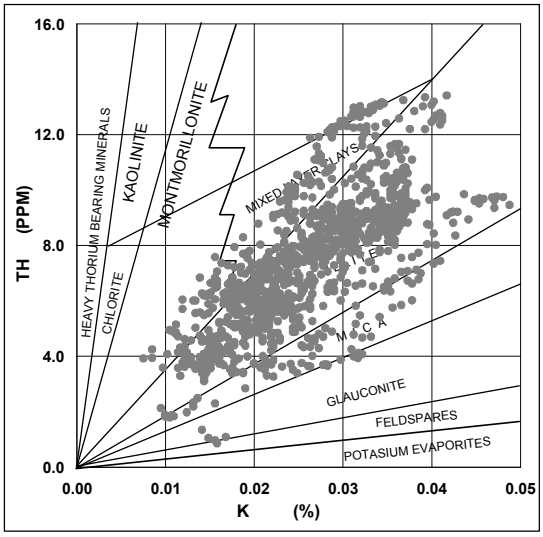

Fig. (10) Th-K crossplot of Lower Bahariya Formation for Bassel-1x well.

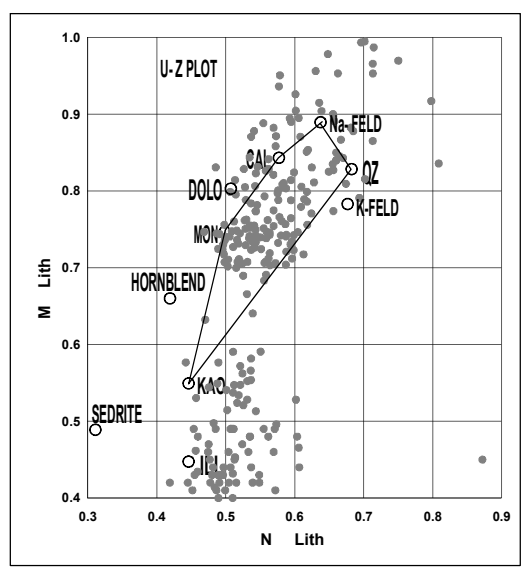

Fig. (9) M lith-N lith crossplot of Upper Bahariya Formation for Bassel-1x well.

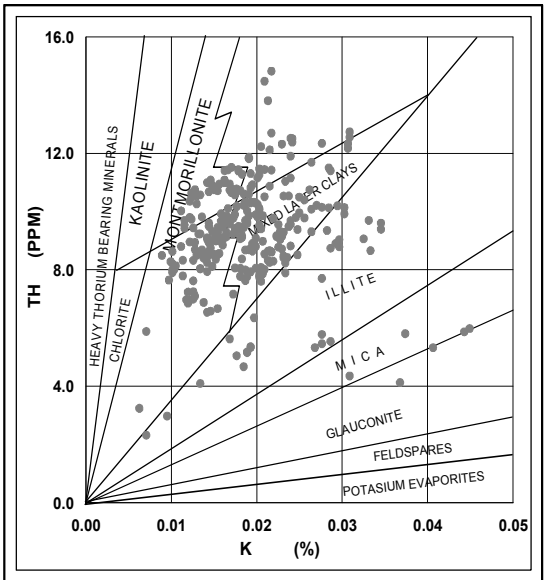

Fig. (11) Th-K crossplot of Upper Bahariya Formation for Bassel-1x well.

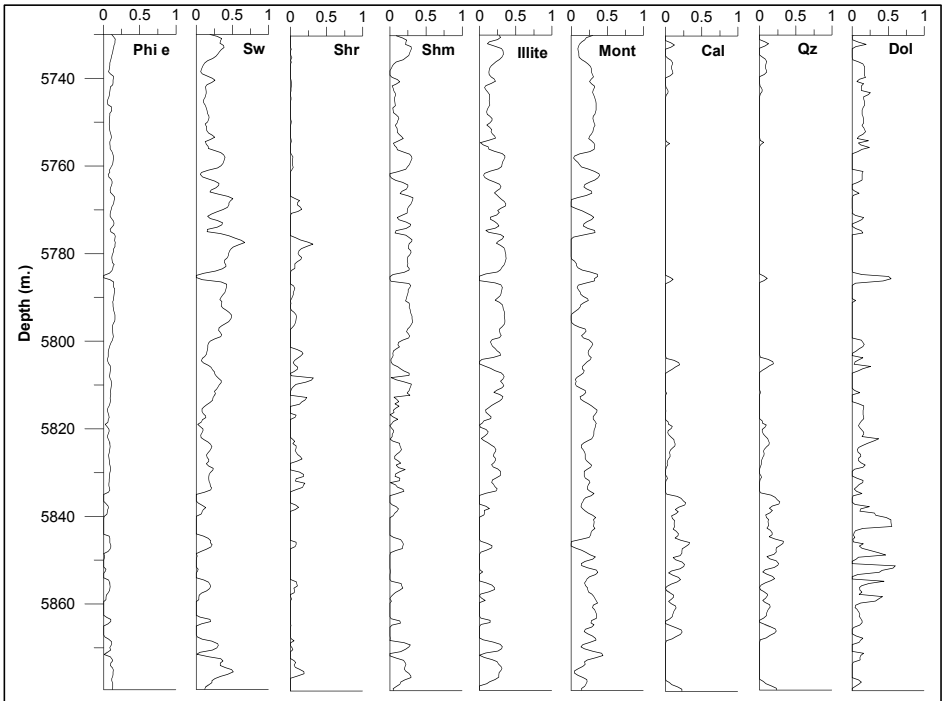

Fig. (12) Impact of mineral types and proportions on the reservoir performance of Upper Bahariya Formation for Bassel-1x well.

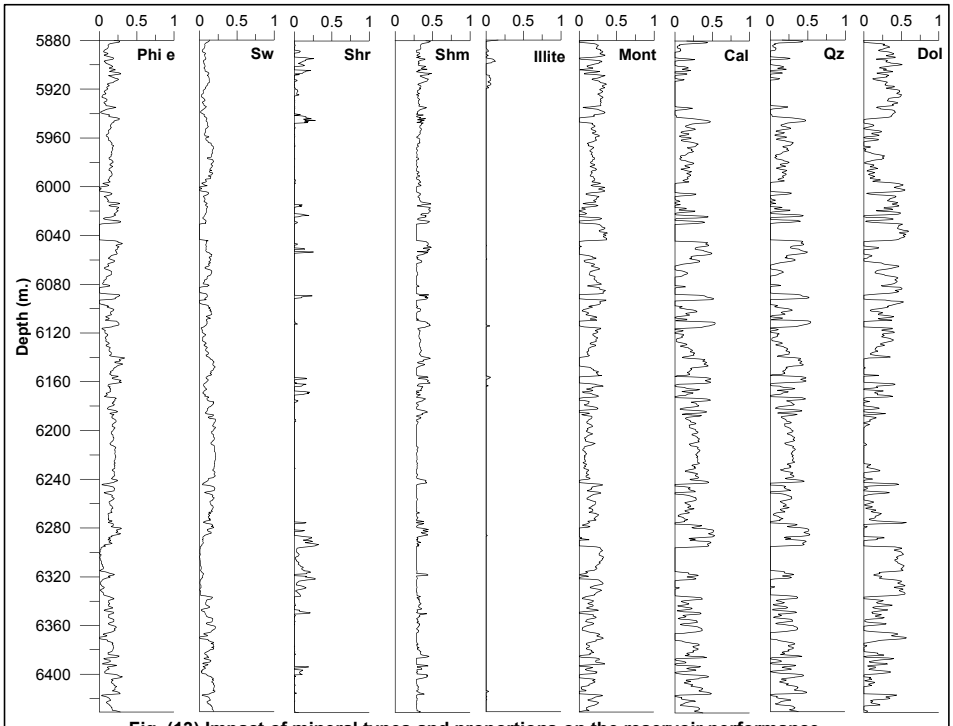

Fig. (13) Impact of mineral types and proportions on the reservoir performance of Lower Bahariya Formation for Bassel-1x well.

Summary and Conclusion

The present work deals with the computerized well - log analysis for Bassel-1x well in the North Western Desert of Egypt. Such an analysis was carried out on Lower and Upper Bahariya Formation where these rock units are very important for petroleum exploration.

The integrated analysis of the available open hole log data is in the form of porosity tool (density, sonic and neutron), gamma ray, natural gamma ray spectroscopy and resistivity logs for invaded and uninvaded zones, these analysis helped in identifying and determining the lithologic and mineralogic components of the rock units of the studied well.

Well log technique started by representing these data in the form of crossplots to facilitate the qualitative and quantitative interpretation for the mineralogical composition of the studied rock units. However, the mineralogical constituents were deduced from these crossplots.

For the Lower and Upper Bahariya Formations, the following minerals are indicated: quartz, calcite, dolomite, illite and montmorillonite. After establishing the mineralogical models of the studied formation, the mathematical equation was applied on the estimated minerals from the crossplot to determine the percentage of each mineral. It was found that the clay minerals (illite and montmorillonite) are found with high percentage in the upper unit while with low ratios in the lower unit of Bahariya Formation.

The effect of these minerals on the different petrophysical parameters of the two rock units is shown by plotting the percentages of these minerals against the different petrophysical properties. From these plots, it was found that the montmorillonite and illite which are found with high ratio in the Upper unit rather than the lower unit caused a major reduction in the effective porosity and then in the hydrocarbon saturation in the Upper Bahariya Formation, while the effective porosity and the hydrocarbon saturation are high in the Lower unit where, these clay minerals didn't have an affect on it. This variation of mineral contents enhanced that the lower rock unit can perform as a reservoir rather than the upper rock unit.

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Comparison between Molecular and Classical Techniques for Identification of *Mycoplasma* species Isolated from Mastitic Ruminants.

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Abstract: 165 cows and 19 buffaloes were examined to detect the *Mycoplasma* mastitis, the result revealed that 114 (69.59%) and 6 (31,57%) were clinically mastitic cows and buffaloes respectively while 51 (30.9%) and 13(68.42%) were apparently healthy cows and buffaloes respectively .On examining the apparently healthy cows and buffaloes, the result were 67 (32.84%) and 18 (34.61%) from subclinically mastitic cows and buffaloes respectively while 137(67.15%) and 34 (65.38%) fro apparently completely healthy. *Mycoplasma* were isolated in percentages of 8.9%, 5.5% from subclinically mastitic cow and buffaloes respectively and in percentages of 12.97%, 12.5% from clinically mastitic cows and buffaloes respectively. *M. bovis* was isolated from 8 (32%) and M. bovigenitalium was in percentage of 7 (28%) and the unidentified *Mycoplasma* was 10 (40%). Isolation of *Mycoplasma* from udder tissue in cows and buffaloes were in percentage of 2 (28.5%) in cows while no *Mycoplasma* isolates were obtained from buffaloes udder tissues. Application of PCR technique on these isolates and some negative samples, these were positive with percentage 100%. On the other hand, 192 sheep and 118 goats were examined. We found that in percentage of 82 (42.7%) and 43 (36.44) from sheep and goats respectively were clinically mastitic. Isolation of *Mycoplasma* was in percentage of 11 (13.41%) and 17 (39.53%) of sheep and goat respectively. Identification of these isolates revealed 8 (29%) was *M. agalactia* isolates and 20 (71%) was unidentified *Mycoplasma* spp. Application of PCR technique on *M. agalactia* isolates which identified by traditional techniques by use specific primers to *M. agalactia* revealed negative results but on using the primer specific to *M. bovis* to the same isolates, it was positive to all isolates 8 (100%).

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Keywords: *Mycoplasma bovis*; *Mycoplasma agalactia*; mastitis; PCR.

1. Introduction:

*Mycoplasma*s can cause many diseases in most species of the animals including human. In small ruminants, they can cause respiratory diseases, mastitis, arthritis, genital diseases and eye lesions. The most important of these diseases are Contagious Caprine Pleuropneumonia (CCPP) and Contagious Agalactia (CA) which are designated by the Office of International Epizooties as list B diseases because of their economic impact on livestock (Nicholas, 2002).

*Mycoplasma*s are distinguished phenotypically from other bacteria by their minute size (125-150 millimicron) and total lack of a cell wall which explains many of the unique properties of the *Mycoplasma*s, such as sensitivity to osmotic shock and detergents, resistance to penicillin, and formation of peculiar fried-eggs shape colonies (Sabry, 2004). *Mycoplasma*s are pleomorphic. They can easily change their shape and may appear as pear-shaped or circular with characteristic "fried egg" shaped colonies.

Mycoplasma bovine, ovine and caprine mastitis are a highly contagious disease that results in milk loss and culling of infected animals(Cree, 2002). Bradley et al. (2007) felt that the current literature did not warrant the widespread screening of mastitis cases for 'exotic' diagnoses, recommending that practitioners keep an open mind in the event of difficult to explain mastitis outbreaks and failures to respond to treatment

Because of their importance in veterinary medicine, and since infection spreads quickly once it established in a herd, it is very important that specific and rapid diagnostic procedures are developed for their detections. Identification of *M. agalactiae* and *M. bovis* by immunofluorescence was laborious and time-consuming. Furthermore, *M. agalactiae* and *M. bovis* possess a particular ability to modify the phase and/or size of the membrane surface proteins, allowing escape of the host's immunodefence (Behrens. *et al*., 1994; Glew .*et al*., 2000).

The use of PCR made the identification of *M. bovis and M. agalactia* quicker compared to the conventional culture methods. In addition the

*Mycoplasma*s can be detected even if the organs or the broth cultures were contaminated with bacteria. (Cardoso *et al*., 2000 and Hirose *et al*., 2001). The risk of false negative test results to a herd can be problematic. Conversely, the risk of false positive test results is reduced in view of the fact that non-pathogenic *Mycoplasma* species rarely cause mastitis (Kirk and Lauerman, 1994).

Incorrect identification by conventional diagnostic methods was recertified by PCR. Isolates from non-typical hosts, i.e. three *M. bovis* strains from small ruminants and two *M. agalactiae* strains from cattle, were characterized by sequencing the 16S and part of the 23S ribosomal RNA genes (Bashiruddin . *et al*., 2005a).

Consequently, this work was planned to clear out the comparison between classical methods and PCR technique in diagnosis of the false negative *Mycoplasma* isolates.

2. Materials and methods

Samples

A total number of 335 and 60 milk samples were collected from udder quarters of examined cows and buffaloes respectively. One hundred and thirty one milk samples were collected from114 clinically mastitic cows which had clinical signs of abnormal secretions of mammary glands containing clots or flakes, with udders showing swelling and hardness and 204 milk samples were collected from 51 apparent healthy one detected by palpation of udder and were subjected to California Mastitis Test (CMT) to detect subclinical mastitis. While a number of 8 milk samples were collected from 6 clinically mastitic buffaloes and 52 from 13 apparent healthy one. On the other hand a total number of 192 milk samples were collected from 82 mastitic and 110 apparent healthy ewes while a number of 118 milk samples were collected from 43 mastitic and 75 apparent healthy goats.

A total number of 80 udder tissues were collected belonged to cows, buffaloes, ewes and goat with numbers 10, 36, 13 and 20 respectively.

Cultivation of *Mycoplasma*: (Razin and Tully, 1983)

For udder tissues:A sample of the udder tissue was seared with a hot spatula to reduce surface contamination and about 0.5 g of the tissue was aseptically removed into a sterile mortar, cut into small pieces by a sterile scissor and grinned with sterile sand, after which 5 ml of broth medium was added.

A part of the mixture was directly plated (Plat 0) was made and about 0.2- 0.3ml was transferred into the broth (Broth 0). By the 3rd day plate (0) and broth (0) were transferred into PPLO plate (1) and broth (1) . On the sixth day, another plating was tried (Plate 3) beside an indirect plating (Plate 2) from the original broth on the 9th day. From Broth (1) an inoculum was made into another broth tube (Broth 2) from which a last plating (Plate 4) was made. The agar plates were inoculated at 37°C under reduced oxygen tension in a CO_2 incubator (5-10% CO_2). The plates were examined for suspected colonies after 48 hours under a stereomicroscope using oblique light and then daily up to 7- 10 days.

For milk samples:

About 1ml of a well mixed milk sample was inoculated in 5ml broth, and a part of the mixture was directly plated (Plat 0) was made and about 0.2-0.3ml was transferred into the broth (Broth 0). By the 3rd day plate (0) and broth (0) were transferred into PPLO plate (1) and broth (1). On the sixth day, another plating was tried (Plate 3) beside an indirect plating (Plate 2) from the original broth on the 9th day. From Broth (1) an inoculum was made into another broth tube (Broth 2) from which a last plating (Plate 4) was made. The agar plates were inoculated at 37°C under reduced oxygen tension in a CO_2 incubator (5-10% CO_2). The plates were examined for suspected colonies after 48 hours under a stereomicroscope using oblique light and then on every other day up to 7- 10 days. Filtration with a syringe filter was used to overcome contaminated samples or fatty samples.

Differentiation between *Mycoplasma* and *Acholeplasma* isolates using the Digitonin sensitivity test (Erno and Stipkovits, 1973 a, b and Freundt, 1973).

Filter paper discs containing 0.02 ml of a 1.5% ethanol solution of digitonin were placed on plates inoculated by the running drop technique with 0.1 ml of cultures. The plates were incubated at 37°C in a moist CO_2 incubator for 3 days, and then examined for the development of inhibition zones around the discs. *Mycoplasma* is digitonin sensitive, while *Acholeplasma* is digitonin resistant.

Biochemical characterization (Erno and Stipkovits, 1973a, b).

Stereotyping of *Mycoplasma* by Growth Inhibition Test (GIT) (Clyde et al., 1984)

Filter paper discs soaked in 20 ul of *Mycoplasma agalactia, Mycoplasma bovigenitalium* and *Mycoplasma bovis* antisera were placed on the inoculated plates by the running drop technique. The plates were incubated at 37°C in CO_2 incubator for 3-7 days. The interpretation was made by observing the zone of inhibition around the antisera discs.

Extraction of DNA by Chemical method using Phenol, *Chlorophorm, Isoamyl:* (Ausubel et al., 2003)

The centrifuged colony pellets were resuspended in 200 µl sterile distilled water to which 200 µl of lysis buffer was added. The mixture was vortexed efficiently then placed in a boiling water bath for 5 minutes. Equal volume of phenol/choloroform/isoamyl alcohol (25:24:1) was added and mixed by vortex then centrifuged at 12.000 rpm for10 minutes. After centrifugation, 3 layers were separated (an aqueous layer containing the DNA, a creamy layer containing the proteinous material, a rosy yellow layer containing phenol). The aqueous layer was transferred to a fresh tube at which an equal volume of phenol/ choloroform/isoamyl alcohol (25:24:1) was added and mixed by vortex then centrifuged at 12.000 rpm for 10 minutes, this step was repeated till the middle proteinous layer disappeared. The aqueous layer was transferred to a fresh tube with the addition of equal volume of choloroform/isoamyl alcohol (24:1) and mixed by vortex then centrifuged at 12.000 rpm for 10 minutes. The aqueous layer was transferred to a fresh tube with an equal volume of isopropanol was added and mixed gently. After storage at -20^0C for 1 hour, the DNA was pelleted at 12.000 rpm for 20 minutes, followed by washing with 70% ethanol and recentrifugation at 12.000 rpm for 10 minutes. The DNA pellet was dried and resuspended in 50µ l deionized distilled water.

Running of PCR: (Riffon et al., 2001)

The amplified reactions were performed in 50 µl volumes in micro amplification tubes (PCR tubes). The reaction mixture consisted of 10 µl (200 ng) of extracted DNA template from bacterial cultures, 5 µl 10x PCR buffer, 1 µl dNTPs (40 µM), 1 µl Ampli Taq DNA polymerase, 1 µl (50 pmol) from each primer pairs (each primer pair was used separately) and the volume of the reaction mixture was completed to 50 µl using deionized distilled water and the thermal cycler was adjusted as follows: For *M.bovis* initial denaturation at 94°C for 2 minutes followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing step at 52°C for 1 minute and extension at 72°C for 150 seconds. A final extension step was done at 72°C for 5 minutes. The PCR products were stored in the thermal cycler at 4°C until they were collected. The amplified product size equals to 227bp for *M. bovis* and loads 10 µl from PCR products.

For *M. agalactia* : initial denaturation at 94°C for 4 minutes followed by 30 cycles of denaturation at 94°C for 60 seconds, annealing step at 57°C for 60 seconds and extension at 65°C for 60 seconds. A final extension step was done at 65°C for 10 minutes. The PCR products were stored in the thermal cycler at 4°C until they were collected.

Screening of PCR products by agarose gel electrophoresis (Sambrook et al., 1989):

The PCR products were electrophoresed in 2% agarose gel using Tris-borate EDTA buffer. The gel containing separated DNA was stained with ethidium bromide and examined under short wave UV transilluminator; Standard marker containing known fragments of DNA either 100 bp or 250 bp ladders was used.

Oligonucleotide primers used for amplification of DNA recovered from *Mycoplasma bovis* isolates:

The PCR amplicone was a part of *M. bovis* DNA sequence, with the following primer sequences these primers amplify a 227 bp fragment. (Yassin et al., 2004).

Forward
5\ GCA ATA TCA TAG CGG CGA AT 3\
Reverse
5\ TCT CAA CCC CGC TAA ACA TC 3\

Oligonucleotide primers used for amplification of DNA recovered from *Mycoplasma agalactia* isolates:The PCR amplicone was a part of *M. agalactia* DNA sequence, with the following primer sequences: these primers amplify a 375bp fragment. (Tola et al., 1996).

Forward
5\AAA GGT GCT TGA GAA ATG GC3\
Reverse
5\GTT GCA GAA GAA AGT CCA ATCA3\

3. Results and Discussion

From the results presented in Table (1) the mastitic cows were 114 out of examined 165 in a percentage of 69.1%. On the other hand the mastitic buffaloes were 6 out of 19 in a percentage of 31.6%, these results were in agreement with those reported by Osman *et al.* (2009). While results in table (2) represent that, out of 204 apparently normal quarters milk samples collected from 51 apparently healthy cows, subclinical mastitis reached 67 with an incidence of (32.84%), and 137 were negative for CMT with an incidence of (67.16%), On the other hand out of 52 apparently normal quarters milk samples of buffaloes, 18 were sub clinically mastitis with an incidence of (34.61%).These results nearly similar with those obtained by Kamelia *et al.* (2008)and Bachaya *et al*. (2005), who reported subclinical mastitis in 32.62 and 26.25% of cows and buffaloes, respectively.

Table (1): Incidence of mastitis among the examined lactating cows and buffaloes.

Udder status / Species	Apparently healthy		Mastitic		Total
	No.	Percentage (%)	No.	Percentage (%)	
Cows	51	30.9%	114	69.1%	165
Buffaloes	13	68.4%	6	31.6%	19

Table (2): Incidence of the subclinical mastitis among the apparently normal quarters cow and buffaloes as detected by CMT.

Animal species	Subclinically mastitic quarters		Normal quarters		Total
	No.	Percentage (%)	No.	Percentage (%)	
Cows	67	32.8	137	67.2	204
Buffaloes	18	34.6	34	65.4	52

% was calculated according to the total number of the examined apparently normal milk samples

Results in table (3) demonstrated that 82 out of 192 examined ewes and 43 out of 118 examined goats were clinically mastitic (42.7% and 36.4% respectively).These results were in agreement with Iqbal *et al*. (2004) .

Table (3): Incidence of clinical mastitis and apparently normal sheep and goats.

Udder / Species	Apparently healthy		Mastitic		Total
	No.	Percentage (%)	No.	Percentage (%)	
Sheep	110	57.3%	82	42.7%	192
Goat	75	63.6%	43	36.4%	118

Table (4) illustrated the subclinical stage the total recovered *Mycoplasma* isolates were 6 (8.9%) from the cows while 1 (5.5%) *Mycoplasma* species isolates were recovered from the buffaloes. On the other hand, the incidence of *Mycoplasma* species isolates that were isolated from the clinically affected quarters milk samples of cows and buffaloes were 17 (12.97%) and one (12.5%) respectively, a similar results obtained by Gonzalez and Wilson (2003).

Table (4): Incidence of *Mycoplasma* in subclinically and clinically mastitic cows and buffaloes (Quarter milk samples).

Quarter status / Species	Subclinically mastitic			Clinically mastitic		
	Examined QMS	Positive QMS		Examined QMS	Positive QMS	
		No.	%		No.	%
Cows	67	6	8.9	131	17	12.97
Buffaloes	18	1	5.5	8	1	12.5

QMS= Quarters Milk Samples

% was calculated according to the total number (No.) of examined quarter milk samples

The results in table (5) revealed in the clinical stage the total number of *Mycoplasma* isolates were 11 (13.41%) from sheep while 17 (39.53%) *Mycoplasma* isolates were recovered from goats, and this agreed with Otlu, (1997).

Table (5): Incidence of *Mycoplasma* in clinically mastitis sheep and goats.

Quarter status / Species	Examined QMS	Positive QMS	
		No.	%
Sheep	82	11	13.41
Goat	43	17	39.53

QMS= Number of quarters milk samples
% was calculated according to the total number (No.) of examined quarter milk samples.

Table (6) showed that *Mycoplasma bovis* isolates causing mastitis in cows and buffaloes were (32%) while these records decreased to (28%) in *Mycoplasma bovigenitalium and* unidentified *Mycoplasma* is 40% respectively these results agreed with that of Biddle *et al*., (2003) and disagreed with Kamelia *et al*.(2008) . On the other hand the results in table (7) illustrated *Mycoplasma agalactia* isolates causing mastitis were (29%) and unidentified *Mycoplasma* were (71%), these results were in agreement with Iqbal *et al*. (2004).

Table (6): Biochemical and serological identification of *Mycoplasma* isolates recovered from clinical mastitic and mastitic cows and buffaloes.

Types of *Mycoplasma* isolates	D.S	U.A	G.F	A.H	Positive isolates (GIT)	
					No.	%
M.bovis	+	-	-	-	8	32
M.bovigenitalium	+	-	-	-	7	28
unidentified *Mycoplasma*	+				10	40
Total					25	100

D.S. = Digitonin sensitivity. U.A. = Urease activity. G.F. = Glucose fermentation. A.H = Arginin hydrolysis
+ve* number of isolates positive to specific antisera by Growth inhibition test (GIT).

Table (7): Biochemical and serological identification of *Mycoplasma* isolates recovered from mastitic milk samples of sheep and goats.

Types of *Mycoplasma* isolates	D.S	U.A	G.F	A.H	Positive isolates (GIT)	
					No.	%
M.agalactia	+	-	-	-	8	29
Unidentified *Mycoplasma*	+				20	71
Total					28	100

D.S. = Digitonin sensitivity. U.A. = Urease activity. G.F. = Glucose fermentation. A.H = Arginin hydrolysis
+ve* number of isolates positive to specific antisera by Growth inhibition test (GIT).

Table (8):Biochemical and serological identification of *Mycoplasma* isolates recovered from udder tissues of cows and buffaloes.

Animal species	No. of examined udder tissue samples	D.S	U.A	G.F	A.H	Positive isolates	
						No.	%
Cows	110	+	-	-	-	2	20
Buffaloes	36					0	0

D.S. = Digitonin sensitivity. U.A. = Urease activity. G.F. = Glucose fermentation. A.H = Arginin hydrolysis
+ve* number of isolates positive to specific antisera by Growth inhibition test.

Table (9): Results of the isolation of *Mycoplasmas* recovered from udder tissues of sheep and goats.

Animal species	No. of examined udder tissue samples	Positive isolates	
		No.	%
Sheep	13	0	0
goats	20	0	0

+ve* number of isolates positive to *Mycoplasma*

PCR and culture methods were applied for the identification of the *Mycoplasma* isolated from bovine milk, to 11 milk samples(10 + 1 reference sample (positive for both PCR and culture). The results showed that out of the 11 samples, only 8 samples were positive for culture while the remaining 3 were negative for culture. On the other hand all 11 samples were positive for PCR using *M.bovis* primers as illustrated in table (10).

Table (10): Results of PCR (*M.bovis*) and culture of 11 milk bovine samples:

Culture (*M. bovis*)	**PCR (*M. bovis*)**		**Total**
	Positive	Negative	
Positive	8	0	**8**
Negative	3	0	**3**
Total	**11**	**0**	**11**

On the other hand the eight *M. agalactia* isolates which identified by cultural and serological methods were negative by PCR using specific *M. agalactia* primers and use reference strain to *M. agalactia* while the same 8 isolates were positive by PCR using *M. bovis* primers as shown in table (11).

Table (11): Results of culture and PCR (*M.agalactia* and *M.bovis*) for 8 milk samples collected from sheep and goat:

Culture (*M.agalactia*)	**PCR (*M. agalactia*)**		**PCR (*M. bovis*)**	
	Positive	Negative	Positive	Negative
Positive	0	8	8	0
Negative	0	0	0	0

As shown in table (11) there is a clear relation between *M. bovis* and *M. agalactia*. However in the present study 8 *M. agalactia* isolates isolated from milk of sheep and goats and identified using traditional techniques and serology, on contrast the application of PCR to these *M. agalactia* isolates, using specific primers for *M. agalactia* revealed negative results,while on using *M. bovis* specific primers on the same isolates the results were positive for all isolates. According to the obtained results and the previous literatures in Egypt it is considered the first record to isolate *M. bovis* from sheep and goats milk, these results were in agreement with (Kumar and Singh, 1984; Chima *et al*., 1986 and Richard *et al.*, 1989) who succeeded to isolate *M. bovis* from sheep and goats.

The incorrect identification by conventional diagnostic methods was recertified by PCR. Bashiruddin et *al*., 2005a reported isolates from non-typical hosts, i.e. three *M. bovis* strains from small ruminants and two *M. agalactiae* strains from cattle, were characterized by sequencing the 16S and part of the 23S ribosomal RNA genes.

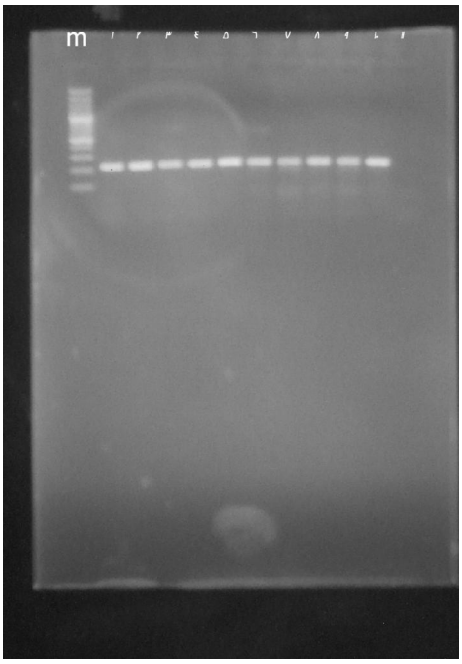

Photo (1): Agarose gel electrophoresis showing amplification of the 227 bp fragments of *M. bovis* from the extracted DNA of *M. bovis* isolates.

Lane M shows the 100 bp- 1.5 Kb DNA ladder.
Lane 1 shows amplification of the 227 bp fragment of *M. bovis* from the extracted DNA of *M. bovis* reference strain
Lane 2-10 showing amplification of the 227bp of *M. bovis*
Lane 11 showing no amplification of the 227bp of *M. bovis* (negative control).

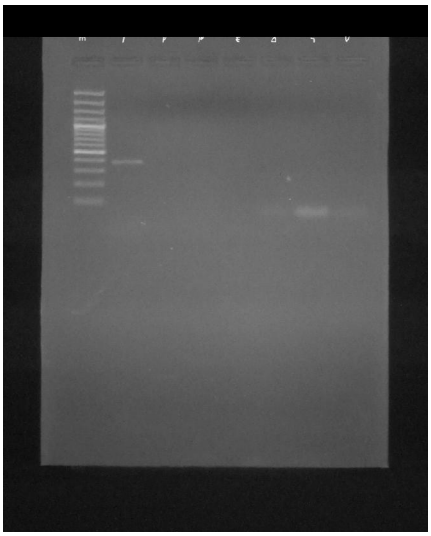

Photo (2): Agarose gel electrophoresis showing amplification of the 375 bp fragment of *M. agalactia* from the extracted DNA of *M. agalactia* reference strain.

Lane M: showing the 100 bp- 1.5 Kb DNA ladder.
Lane 1: *M. agalactia* reference strain
Lanes 2-6 showing no amplification of the 375 bp fragment of *M. agalactia* from the extracted DNA of other *Mycoplasma* isolates.
Lane 7 shows no amplification of the 375 bp fragment of *M. agalactia* (negative control).

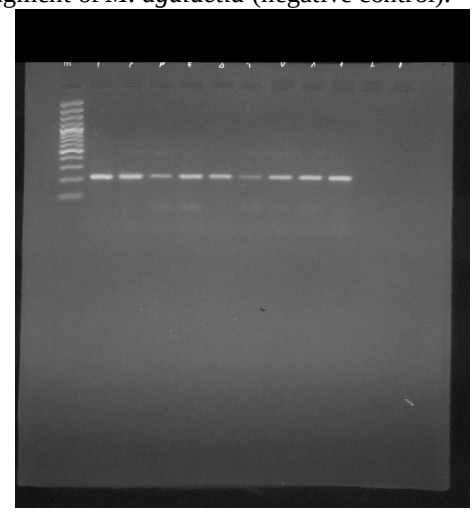

Photo. (3). Agarose gel electrophoresis showing amplification of the 227 bp fragment of of *M. bovis* from the extracted DNA of *M. bovis* reference strain.

Lane M showing the 100 bp- 1.5 Kb DNA ladder.
Lane 1: *M. bovis* reference strain.
Lanes 2-9 showing amplification of the 227 bp fragment of *M. bovis* from the extracted DNA of other *Mycoplasma* agalactia (which gives positive culture and negative PCR agalactia).
Lane 10 showing no amplification.

4. Conclusion:

In conclusion, *Mycoplasma*s isolates were grown slowly and were difficult to culture. Traditionally, very complex media had been used for culture, based on rich growth media have recently been found to be inhibitory in some cases. Incubation and observation should continue for 7-10 days before the plates were recorded as negative but false-negative results were common due to low numbers of organisms in the sample, or the fragility of *Mycoplasma* itself. Although serological methods are easier to perform and less costly, however, they are also generally non-specific, insensitive, and retrospective. PCR-based technology for *Mycoplasma* yields the highest level of sensitivity and specificity. The detection of *Mycoplasma* spp in cattle, buffaloaes, sheep and goats by polymerase chain reaction (PCR) was based on the *in vitro* amplification of the highly-conserved 16S rRNA gene, so using PCR technique to differentiate between *M. bovis* and *M. agalactia* because of the close relation between each other and this technique is rapid, sensitive and specific. Recommended future work to apply PCR technique directly on milk samples and udder tissues to make a comparison between results of culture and PCR.

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Molecular and Virulence Characterization of *Escherichia.coli* strains Isolated from Persistent Bovine Mastitis.

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Abstract: Four hundred and fifty lactating cows were examined according to the clinical observation and the California mastitis test, 181 were clinical mastitis with the percentages of 40.2%, and revealed 57 *E.coli* isolates, the incidence of clinical mastitis is higher in hind quarters (63.97%) than the fore quarters (36.02%). Serotyping of *E.coli* revealed 8 different serovars of *E.coli* according to somatic antigen O55 (19.2%), O111 (15.8%), O124 (12.3%), O119 (12.3%), O114 (10.5%), O26 (7%), O157 (7%) and O44 (3.5%), in addition, (12.2%) of isolated *E.coli* strains could not be serologically identified by the available antisera. The incidence of recurrent *E.coli* mastitis, 26.3% (15 of 57) occurred in 5 of 56 quarters 8.9% of 5 cows, the most *E.coli* serogroups recovered from recurrent *E.coli* mastitis from 5 quarters of 5 cows were O55, O119, O111, and O157. The adherent and invasive property were the most common factors in *E.coli* serogroups (O55, O119, O111 and O157) which were isolated from recurrent mastitis and give positive results with (*eae*A) gene but it is less in *E.coli* serogroups(O124, O114, O26 and O44) which give negative results with (*eae*A) gene**.**

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Key words: Recurrent mastitis- adherent *Escherichia coli*-intimin

1. Introduction:

Mastitis is considered the most costly disease in dairy herds due to discarded milk and lowered milk production for approximately 80% of costs associated with mastitis, treatment costs, veterinary fees, labor costs early culling and death (Miller *et al*., 1993). Lowered milk quality due to increased somatic cell count (SCC) in the milk decreases shelf life of milk and cheese making quality (Klei *et al*., 1998 and Ma *et al*., 2000).

The importance of mastitis in public perception should not be over looked. The general public is more and more concerned with animal welfare, possible antibiotic residues in the milk and a disease such as mastitis that can cause severe distress to the cow shouldn't be ignored(Bradely, 2002). Mastitis is considered of vital importance due to its association with many zoonotic diseases in which milk acts as a source of infection (APHA., 1993).

E.coli is one of the most frequently isolated pathogens from both clinical and chronic infection, it was more severe than the other bacterial causes and it tended to be more severe in early lactation and during the housing period, resulting in inflammation that ranges from sub-acute to per-acute. Necrosis of the mammary epithelium occurs during severe, naturally occurring clinical *E.coli* mastitis, as well as during severe experimental *E. coli* mastitis. In moderate cases of *E. coli* mastitis, there is minimal alveolar tissue damage (Bradley and Green, 2001).

Recurrent clinical mastitis caused by *E. coli* in a cow that express persistent intra-mammary infection (IMI) is known to exist in the same quarter can be caused by a persistent IMI or may be associated with recurrent IMI. (Lipman *et al*., 1995).

Adherence of microorganisms to the host cells is the first step in colonization on the host surface (Finlay and Falkow, 1997). Invasion and adhesion are important virulence mechanisms in the bacterial infection, Therefore persistent bacterial infection generally involves adhesion, invasion and intracellular survival (Finaly and Cossart, 1997). Pathogenic *E.coli* which can cause persistent intramammary infection have several fimbrial and afimbrial adhesions that mediate adhesion to host epithelial cell through the cell surface compound

like proteins, glycolipids and carbohydrates (Le Bouguenec, 2005).

The present study was directed to detection and characterization of virulent E.coli pathogen recovered from mastitic milk with special reference to recurrent mastitis.

2. Materials and methods

Samples:

A total of 450 milk samples were collected from cows from 4 farms in Kafrelsheikh and Dakahlia Governorates. All samples were examined for mastitis according to clinical observation and California mastitis test as shown in Table (1).

Bacteriological examination of milk samples (Quinn et al., 2002):

The collected milk samples were incubated aerobically at 37°C for 18-24 hours, then centerifugated at 3000 rpm for 20 minutes. The cream and supernatant fluid were discarded. The sediment was streaked onto blood agar, MacConkey agar and EMB agar. The inoculated plates were incubated aerobically at 37ºC for 24-48 hours and examined for bacterial growth.

Identification of isolates:

Pure cultures were prepared from all suspected colonies. The shape, size and type of colonies either lactose or non-lactose fermenting colonies onto MacConkey's agar and blood agar were recorded. Gram's stained films from the purified isolates were made on clean slides films to be examined microscopically for detection their stain reaction and morphological characters. Gram negative rood shape bacilli or coccobacilli, with parallel sides and round ends. Such colonies were picked on slope agar for preservation of isolates and for further studies (Koneman et al., 1995).

Biochemical identification:

Members of this group were initially identified by their catalase positive, oxidase negative and gram negative bacilli in Gram stained smears. Further identification was done according to Quinn et al. (1994).

Serological identification of *E.coli*

Serotyping of *E.coli* isolates was performed according to (Edwards and Ewing, 1972).

Fifty seven isolates of *E.coli* from mastitic cows were sub-typed by using 8 polyvalent and 43 monovalent "O"antisera. All isolates were taken from mastitic cows showing sever manifestation. Each isolate was first tested for its agglutinability of the diagnostic polyvalent "O" antisera, which are intended for use by slideagglutination technique. Once the pathogenic type has been indicated by the use of polyvalent sera, further serogrouping was made with the appropriate "O" monovalent antisera.

Identification of K99 pilus in *E.coli* strains:

From *E.coli* suspected colonies from each culture plate were selected and used for K99 pilus detection.Proposed positive *E.coli* samples were collected and cultured on Minica Iso Vitalex medium (Guinee *et al*., 1977). Colonies from each cultured plate were selected and subculture on Minica Iso Vitalex agar plate for purification .Plates were inoculated for 16-18 hr. at 37ºC. All selected colonies were smooth translucent circumscribed, completely separate colonies. These colonies were subjected for K99 pilus antigen detection by slide agglutination test using ready made international trading diagnostic serum.

Extraction of DNA from the bacterial isolates according to Sambrook *et al.* (1989):

Bacterial culture was grown in 5 ml Tryptic Soy broth (TSB) and then 1.5 ml of the culture were microfuged at 6000 rpm for 2 minutes. Pellets were resuspended in 567µl Tris-EDTA by repeated pipetting. 30µl of 10% SDS and 3µl of 20 mg/ml proteinase K were added, mixed and incubated for 1 hour at 37°C. 100µl of 5M NaCl were added and mixed thoroughly. 80µl CTAB/NaCl solution were added, mixed and incubated for 10 minutes at 65°C. Equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) was added, mixed and microfuged for 5 minutes. The supernatant was transferred to a fresh tube then 0.6 volume of isopropanol was added and mixed gently. After storage at 20°C overnight, the DNA was pelleted at 14,000 xg/min for 20 minutes, followed by washing with 500µl 70% ethanol and recentrifuged for 10 minutes at 14,000 xg/min. The supernatant was discarded carefully and the precipitate was dried briefly in laminar air flow, and resuspended in 20µl sterile distilled water.

PCR amplification of the extracted DNA from the bacterial isolates using species-specific primers according to Riffon *et al.* (2001):

All reactions were carried out in a final volume of 50µl in micro amplification tubes (PCR tubes). The reaction mixture consisted of 1µl (200ng) of the extracted DNA template from the bacterial cultures 5µl 10X PCR buffer (75 mM Tris-HCl, pH 9.0, 2 mM $MgCl_2$, 50 mM KC1, 20 mM $(NH_4)_2SO_4$), 1µl dNTPs (40 uM), 1µl (1 U AmpliTaq DNA polymerase), 1µl (50 pmol) from the forward and reverse primers (Eco223-Eco455) primer pairs, each

primer pair used separately and the volume of the reaction mixture was completed to 50µl using DDW. 40µl paraffin oil was added and the thermal cycler was adjusted as follows:

Initial denaturation at 94°C for 4 min followed by 30 cycles of denaturation at 94°C for 45 seconds, annealing step at 65°C for 1 min and extension at72°C for 2 min. A final extension step was done at 72°C for 10 min. The PCR products were stored in the thermal cycler at 4°C until they were collected.

Multiplex PCR assay for the simultaneous detection of intimin gene (encoded by *eae*A) in the extracted DNA of *E.coli* according to Paton and Paton (1998):

The amplified reactions were performed in 50µl volumes in PCR tubes. The reaction mixture consisted of: 1µl (200µl) of the extracted DNA template from the *E.coli* isolates, 5µl 10X PCR buffer [75 mM Tris-HCl, pH 9.0, 2 mM $MgCl_2$, 50 mM KCl, 20 mM $(NH_4)_2SO_4$], 1µl dNTPs (40µM), 1µl (50 pmol) *eae*A primer, 1µl (50 pmol) *eae*A primer, 1µl (1.5 U) Ampli Taq DNA polymerase and 38µl double distilled water.

Then the reaction mixture was overlaid with 40µl paraffin oil and subjected to 35 PCR cycles, each consisting of 1 min. of denaturation at 95°C; 2 min of annealing at 65°C for the first 10 cycles, decrementing to 60°C by cycle 15; and 1.5 min. of elongation at 72°C, incrementing to 2 min from cycles 25 to 35. After the final cycle, the preparation was kept at 72°C for 10 min to complete the reaction. The PCR products were stored in the thermal cycler at 4°C until they were collected.

Agarose gel electrophoresis according to Sambrook *et al*. (1989):

Electrophoresis grade agarose was prepared of IX electrophoresis buffer (TAE) to reach the required concentration 2% and volume (to make 4 mm thick layer). The mixture was heated in a microwave with periodical agitation to check melting degree in between bursts. It was allowed to cool to 70°C, and then the ethidium bromide (0.5µg/ml) was added and mixed thoroughly.

The PCR product and the suitable molecular weight marker were mixed with the loading buffer (15µl PCR product + 3µl loading buffer) followed by loading of the samples into the gel, then the tank gel was closed and attached with the power supply. The running parameters were 1-5 Volts / cm of the tank length and for many gels, 5-20 Volts / cm. Bromophenol blue was allowed to run 2/3 of the gel length before termination of the run, and after the run was stopped, the gel was transferred to the trans-illuminator to observe the amplified DNA on the gel in comparison to the molecular weight marker.

Table (1): Oligonucleotide primers used for amplification of *eae*A gene from the DNA of *E.coli* isolates and primers for detection of mastitic *E.coli* isolates

Primers	Sequence(5 -3)	Specificity	Amplicon size	Annealing temp.
Eco 223-f	ATC AAC CGA GAT TCC CCC AGT	*E.coli*	231 bp	64°C
Eco 445-R	TCA CTA TCG GTC AGT CAG GAG			
***eae*A-F**	GAC CCG GCA CAA GCA TAA GC	Intimin gene (encoded by *eae*A)	384 bp	65°C
***eae*A-R**	CCA CCT GCA GCA ACA AGA GG			

Congo red (CR) binding activity (Berkhoff and Vinal, 1986):

E.coli strains were cultured onto Congo red medium. The reaction is best seen after 24 hr. of incubation at $36C^o$ and then left at room temperature for additional 3 days (not to exceed 4 days).

Adherence assays (Donnenberg and Nataro, 1995):

Ten ml of overnight bacterial cultures in peptone water (containing 1% D-mannose) was inoculated into cover slip – containing 24 – well plates which had been seeded with 5×10^5 HEp-2 cells 48h before. Cultures were incubated at 37°C for 3h. The cells were washed. Fresh RPMI 1640 was added and then the cells were incubated for other 3hours, the cells were fixed with 3% formalin, and cultures were stained with Giemsa solution. The adhesion was determined by light microscopy covering the whole slide. Bacteria were recorded as adhered if a cluster of at least 10 bacteria adhered per HEp-2 cell.

Invasion assays (Tang *et al.*,1993 and Janda and Abbott, 1998):

Ten ml of bacterial culture in peptone water was incubated with HEp-2 cells usually for 2-3h to allow attachment and penetration of the epithelial cell. Gentamicin, (which is unable to penetrate mammalian cells) was added to eliminate extracellular bacteria, and the tissues cell sheet was again incubated 24 hr. to allow multiplication of the bacteria that had invaded. The cells sheet was washed, fixed and Giemsa stained for visual examination of internalized bacteria. The bacteria can be seen in Giemsa– stained preparations as dark blue forms within, and usually filling the cytoplasm, or within cytoplasmic vacuoles.

Antibiogram assay for the local isolates recovered from the examined cows:

The test diffusion technique was applied according to Finegold and Martin (1982).

3. Results and Discussion:

From the results presented in Table (2) Out of 450 lactating cows examined according to clinical observation and California mastitis test, 181 were clinical mastitis with the percentages of 40.2%, these results nearly similar to that reported by Bartlett *et al*. (2001) with an incidence of 38.7%.

Out of 56 quarters affected, the prevalence was higher in hind quarter (63.97%) (116 of 181) than the fore quarters (36.02%) (65 of 181), this result nearly similar incidence was recorded by Wadhwa *et al*. (1996) who reported that out of 93 quarters affected by clinical mastitis, the prevalence was higher in hind quarters (57%) than the fore quarters (43%) and related these result to higher contamination of hind quarters with urine and feces. *E.coli* was the most common cause of clinical mastitis, accounting for 57 isolates in percentages 31.4% of all isolates. Fifty seven cases of clinical *E.coli* mastitis occurred in 56 quarters of 49 cows, Similar results were observed by Aziz (2002) who found that *E.coli* was predominant isolate (37.7%) and Bradley and Green (2001) who demonstrated that the most common cause of clinical mastitis was *E.coli* (34.7%).

Table (2): Incidence of clinical *E.coli* mastitis among lactating cows:

Farm Locality	No. of cows in farm	Clinical mastitis cases		Quarters affected				Clinical *E.coli* cases	
				Hind		Fore			
		No.	%	*No.*	%	*No.*	%	*No.*	%
1	115	56	48.60%	36	64.28%	20	35.71%	15	26.70%
2	80	32	40%	20	62.50%	12	37.50%	12	37.50%
3	135	50	37%	32	64%	18	36%	14	28%
4	120	43	35.80%	28	65.11%	15	34.88%	16	37.20%
Total	**450**	**181**	**40.20%**	**116**	**63.97%**	**65**	**36.02%**	**57**	**31.40%**

The results of the serogrouping of 57 *E.coli* isolates from clinical mastitis is indicated as shown in Table (3), in which distribution of *E.coli* serogroups according to somatic "O" antigen and capsular (K99) antigen. The most prevalent serogroups recovered from mastitic cases were O55 (19.5%), O111 (15.8%), O124 (12.3%), O119 (12.3%), O114 (10.5%), O26 (7%), O157 (7%) and O44 (3.5%).

While mean, 7 (12.2%) isolates were untypable with the available antisera, this observation is in agreement with Aziz (2002) who cited that *E.coli* recovered from mastitic cases belong to different serogroups and nearly in agreement with Correa and Marin (2002) who determined (12) O-serogroups, belong to the classical enteropathogenic serogroups (O26, O55, O11, O114, O119, O125, O126, O127, O128, O142, O158) represented 77.4% of the isolates. However, 12.5% of the obtained *E.coli* isolates were found to be untyapable. Similar observation was recovered by Lipman *et al*. (1996) who found that 7 *E.coli* isolates out of 30 (23.33%) were untypable. This could be attributed to the presence of other serogroups against with no diagnostic antisera were available.

Also it's clear from Table (3) *E.coli* strains O55, O111, O119 and O157 were given positive results with slide agglutination test for detection K99 pilus, while O124, O114, O26 and O44 give negative results,this result agree with Galone and Le-Roux (2001) which found the strains O111 and O119 isolated from mastitic cow contain K99 antigen.

Table (3): Serotypes of *E.coli* isolated from clinical mastitis:

Total *No.* of *clinical E.coli* isolates	**Serogroup**	***No.***	***%**	**K99**
57	O55	11	19.2%	+
	O111	9	15.8%	+
	O124	7	12.3%	_
	O119	7	12.3%	+
	O114	6	10.5%	_
	O26	4	7%	_
	O157	4	7%	+
	O44	2	3.5%	_
	Total	**50**	**87.6%**	
	Untyable	**7**	**12.3%**	

*The percentage was calculated according to the number of *E.coli* serogroups and total number of clinical *E.coli* isolates (57).

Recurrent *E.coli* mastitis results are outlined in Table (4). Five quarters of five cows experienced more than cases of clinical mastitis, recurrent *E.coli* mastitis occurred in 8.9% (5 of 56) of all affected quarters (56).One quarters experienced four cases of clinical *E.coli* mastitis of each one , two quarters experienced two cases for each one and one quarters experienced three cases for each one .The recurrence mastitis is high in hind quarter 80% (4 of 5) than forward one 20 (1 of 5).The most *E.coli* serogroups recovered from recurrent *E.coli* mastitis were O55, O119, O111, and O157 as shown in Table (4). The same serogroups of *E.coli* recovered from one quarter in more cases of episodes. Of all cases of clinical *E.coli* mastitis, 26.3% (15 of 57) occurred in quarters that experienced two or more cases of clinical *E.coli* mastitis in persistently infected quarters as measured by recurrence of clinical mastitis is much higher than those in previous reports (20.2%) (Bradley and Green, 2001). This apparent shift in behavior could be indicative of either a change in the susceptibility of the bovine population to persistent infection or a change in the behavior of *E.coli*.

Table (4): Incidence of recurrence *E.coli* mastitis

Quarters affected	No.of times affected	*No. of *E.coli* isolates from quarters	*E.coli*serogroup isolates
Left hind	4	4	O55
Right hind	3	3	O157
Left forward	2	2	O111
Left hind	2	2	O157
Left hind	4	4	O119
Total	**15**	**15**	

*No of *E.coli* isolates from quarter= (No. of quarters affected × No. of times affected inquarters).

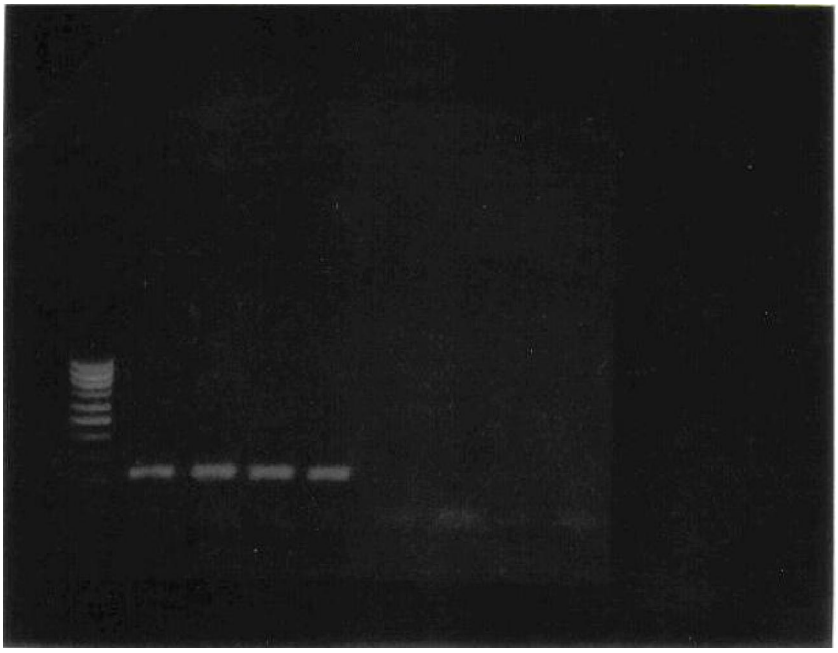

Photo (1): Agarose gel electrophorasis showing the specificity Eco 223 and Eco 455 primers. Amplification of 232 bp fragment was observed with extracted DNA of *E.coli* (Lanes 1, 2, 3, and 4), lane M showing marker.

The extracted DNA of 8 *E.coli* serogroups isolated from clinical *E.coli* mastitis were tested with multiplex PCR using primers for intimin gens (*eaeA*). Results observed in Table (5) and Photo (2) revealed positive amplification of 384 bp fragment of intimin gene from the extracted DNA of 4 *E.coli*serogroups isolates belong serogroups (O55, O119, O111 and O157) in lane (3,4,7 and 8 respectively). This observation is similar to those reported by Kobori *et al*., (2004) who study 31 *E.coli* strains isolated from mastitic cows. He found that *E.coli* serogroups (O111, O119, and O55) were positive *eae*A gene but serogroups (O125, O86 and O142) were negative *eae*A gene.

No amplification could be observed with extracted DNA of other serogroups (O124, O114, O26 and O44) in lane (1, 2, 5 and 6 respectively).

Table (5): Characterization of 8 *E. coli*serogroup isolates recovered from milk samples of mastitic cow by multiplex PCR assays for intimin (eaeA) gene:

***E.coli*serogroup**	**Multiplex PCR for *eae*A gene**
O55	+
O111	+
O124	–
O119	+
O114	–
O26	–
O157	+
O44	–

Table (6): Congo red binding activity of *E.coli*serogroups isolated from mastitic cow:

Mastitic*E.coli*serogroup	**CR binding activity**
O55	+
O111	+
O124	-
O119	+
O114	-
O26	-
O157	+
O44	-
Total positive %	**50%**

Photo (2): Ageros gel electrophoresis showing the amplification of 384 bp fragment of intimin (eaeA) gene from the extracted DNA of *E. coli*serogroup (O55, O119, O111 and O157) in lane (3,4,7 and 8 respectively) while other serogroup (O124, O114 and O44) give negative results with intimin (eaeA) gene in lan (1,2,5 and 6 respectively), lane M showing marker.

Table (6) demonstrated the Congo red activity of 8 *E.coli*serogroups isolates from mastitic cows.The (CR+) was observed in *E.coli*serogroups(O157,O111,O55 and O119) but other serogroups give (CR-).

As shown in Photos. (2,3) , out of 4 *E.coli*serogroups (O157,O111,O55 and O119) isolated from recurrent clinical *E.coli* mastitis which give positive results with (*eae*A) gene could adhere to Hep-2 cells..From this results it clear that *E.coli*serogroups which contain contains intmine (*eae*A) gene could be adhere to Hep-2 cells. All isolates gave 100%. These results were nearly similar to those reported by Wieler *et al.* (1998) who found that the isolates possessed adhesion at percentage 88.1%.

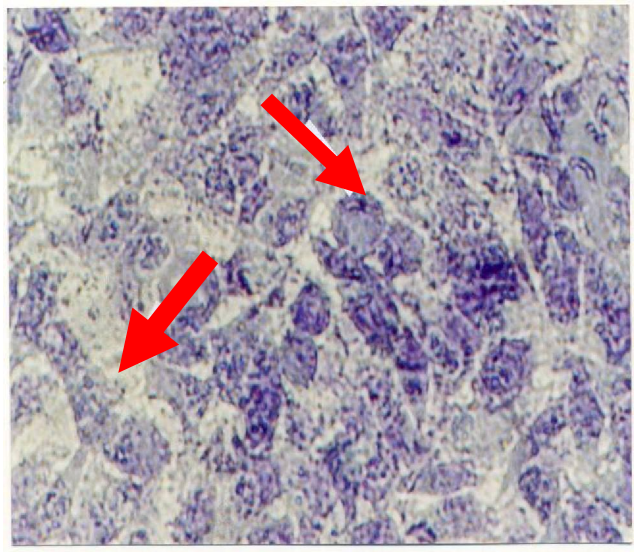

Photo (1): Normal rounded Hep2-cell complete sheet (control) (Geimsa – X:100)

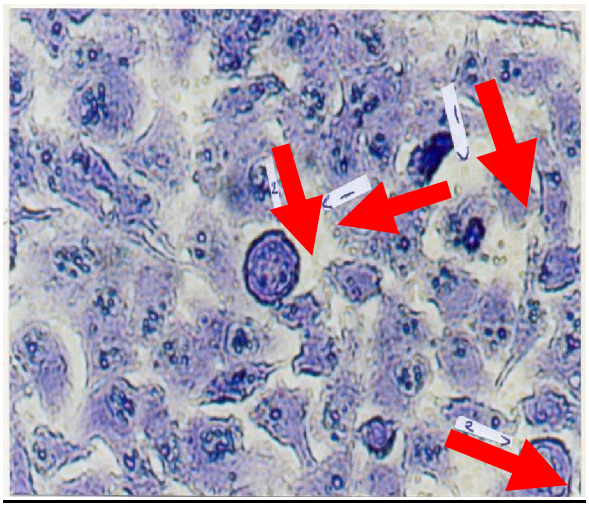

Photo (2): Vaculation of the Hep2- cell sheet & incomplete adhesion of *E.coli* cells after 3 hours (Geimsa– X: 100)

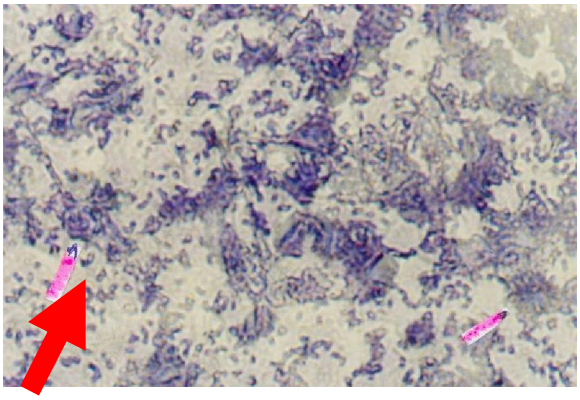

Photo (3): Adhesion of *E.coli*cells to Hep2- cells after 7 hours (Geimsa– X:100)

As shown in Photo (4&5) *E.coli* which isolated from recurrent clinical *E.coli* mastitis from cows

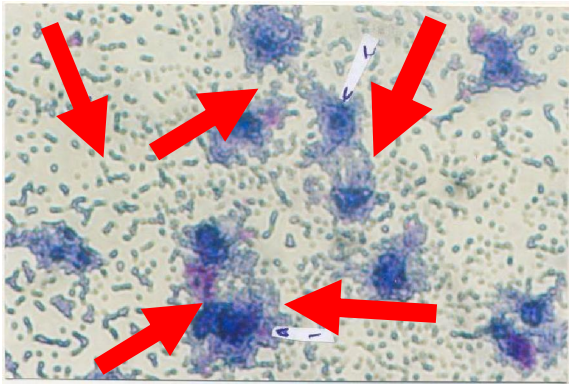

Photo (6): The separated cell sheet and high invasion of *E.coli* cells for Hep2- cells after 24 hours (Geimsa– X:100)

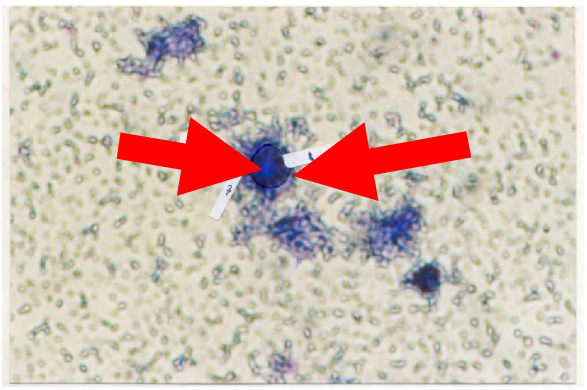

Photo (7): Shows complete invasion of *E.coli*to Hep2- cells &the changes of Hepto cell morphology. (Geimsa– X:100)

As showed in Table (7) . *E.coli* is highly sensitive to nalidixic acid, pefloxacin, gentamycin, sulbactam+ampicillin , cefoperazon flucloxacillin , ampicillin , amoxicillin + clavulonic acid andofloxacin, these results simulate what was reported by Jha *et al* (1994) who found that *E.coli* isolates recovered from clinical mastitis were highly sensitive to genyamycin, kanamycin and ampicillin.

Sympol	O26	O119	O124	O157	O55	O44	O114	O111
TOB	Int	S	R	S	R	S	Int.	Int.
AMC	R	R	SS	S	Int.	S	SS	R
CEP	R	R	Int.	R	R	R	SS	R
CAZ	R	Int.	S	R	R	R	R	R
NA	R	SS	SS	Int.	R	Int.	Int.	R
FL	S	S	S.	R	S	S	SS	S
PEF	Int.	SS	Int.	SS	R	R	SS	R
SAM	R	R	S	SS	R	Int.	S	R
OFX	R	R	Int.	R	Int.	Int.	Int.	R
AM	R	Int.	S	R	R	SS	SS	R
CN	R	SS	R	R	S	R	R	S

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Hydrochemistry and levels of some heavy metals in samples of Ibeshe, Lagos Lagoon Complex, Nigeria

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ABSTRACT: The concentration of Iron, Copper, Chromium, Nickel, Lead, Manganese, Arsenic, Cadmium and Zinc were determined in the surface water, sediments and fish samples (*Chrysichthys nigrodigitatus*) of an industrial effluent receiving water in Ibeshe, Lagos Lagoon Complex between February and June, 2009. In assessing the impact of effluent discharge on the lagoon, Water and fish samples result were compared with the WHO/FEPA standard while the sediments results were compared with the results for unpolluted sediment. The average levels of heavy metals found in surface water, sediment and fish samples were as follows: surface water; 0.293mg/l for Fe, 0.177mg/l for Cu, 0.107mg/l for Pb, 0.213mg/l for Cr, 0.177mg/l for Mn, 0.233mg/l for Ni and <0.10mg/l for Cd. Sediment; 85303.33µg/g for Fe, 53.967µg/g for Cu, 38.35µg/g for Pb, 110.183µg/g for Zn, 93.88µg/g for Cr, 274.967 µg/g for Mn, 1.017 µg/g for As, 67.4 µg/g for Ni and 1.00µg/g for Cd. Fish sample; 4.263 µg/g for Fe, 8.229µg/g for Cu, 1.967µg/g for Pb, 11.338µg/g for Zn, 1.329µg/g for Cr, 1.513µg/g for Mn, 4.046µg/g for Ni and 0.458µg/g for Cd. The concentration of Pb and Ni in surface water were higher than WHO / FEPA limits, while Cd, Zn, Cr and As were found below FEPA limit. Fe, Cu, Pb, Cd and Zn were all higher in concentration when compared with the values of unpolluted sediment. Consequently, the concentration of Zn, Cr, Cd in fish were below the FEPA limit. Water quality of Ibeshe were typify of alkaline pH (8.90 - 9.00), high Dissolved Oxygen content (4.20 -7.80mg/l), Turbidity (24.8 – 156NTU) and freshwater salinity values (0‰). The findings reported in this study would be expected to serve as baseline level for future heavy metal pollution status of the Ibeshe, Lagos Lagoon area.

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1.0 Introduction:

Increase in industrial development and population of the cities have been recognized as a major sources of pollution throughout the world, most especially in the developing nation, {WHO, 1982}. The discharged of effluent by small scale industries have been equated to that of the sewage of a large city (Agarwark, 1999). Industry is recognized to be very important for economic development due to its capability to create employment for large number of people and her ability to reduce poverty.

Meanwhile, the operations or processes of some of these industries are capable of generating enormous volume of toxic waste water that could be detrimental to the environment.

Industrial untreated waste water/ effluent discharge into the environments are characterized by; foul smell and bad odour, high level of organic matter, increase in BOD, COD, TDS, TSS, and metals (Heavy metals) near point of disposal(EPA,1974). Also known to be affected is the dissolve oxygen (DO) of the water which will in turn poses a great threat to the survival of aerobic living organisms in the aquatic environment.

Aquatic environment is one of the receiving ends for these waste water components, at the same time it is also among the last destination for the effluent before some of its components such as organochlorine compounds and metals (trace metals) plough back into the food chains, through bio-accumulation in plankton to fishes and finally bio-magnified in man.

The concentration of industries at Ibeshe and the discharge of their effluents into the water body is a thing of concern, as it may cause heavy metals contamination which may have devastating effects on the ecological balance of the aquatic environment.

In light of this, assessment of levels of some heavy metals and hydrochemistry of Ibeshe, an industrial effluent receiving water body in the north of Lagos Lagoon becomes imperative, not only because of the threat of heavy metals to public water supplies, but also the damage caused to the aquatic life. This assessment therefore entails the monitoring of the aqueous phase (surface water), biota (fish

tissues) and sediments, (Benson et al., 2007, Saad Al-Sulami et al., 2002, Kakulu & Osibanjo, 1992).

2.0 Materials and Methods

Location: $6^{o}32'54.65"$ N, $3^{o}28'08.66"$ E and Elevation 0ft.

The study area characterized in this study was Ibeshe (North) Lagos Lagoon water front. The water front received the effluent from industries such as textile mills (NICHEMTEX), Foods industries and others situated at the Ibeshe community area, Ikorodu axis of Lagos state, Nigeria. Three (3) sampling points were chosen to represent the entire study area (activity points) (fig.1 above).

2.1 Sample collection

Surface water, fish and sediment samples were collected with the aids of water sampler, cast nets and van-veen grab respectively (APHA, 1995) from the three sampling stations along the Ibeshe (Ikorodu) Lagos lagoon. Samples were collected once in a month for five consecutive months, from February to June 2009, representing the late dry season and early raining season in the three sampling stations.

Water and sediment samples were kept in sterile polypropylene bottles and black polythene bags respectively. These water samples were preserved with concentrated Nitric (HNO_3) acid and stored at 4°C in an ice pack prior to the time they were analyzed.

The fish samples were kept in the ice pack from sampling station and later stored in the refrigerator (4°C) before analysis.

2.2 Physiochemical analysis:

Temperature, pH, Conductivity, turbidity, and salinity of the water samples were measured in-situ using Horiba U10 while dissolved oxygen was determined using Winkler method and alkalinity through titration in the laboratory (APHA, 1989).

2.3 Metal analysis:

The levels of Fe, Cu, Pb, Cd, Zn, Cr, As, Mn and Ni metals in different surface water, sediment and fish samples were analyzed by Atomic absorption spectrophotometer using Buck scientific 200A model Atomic Absorption Spectrophotometer (AAS).

50ml of each of the water samples were digested separately using 25ml of ratio 1:3 of concentrated HNO_3 and HCl at 105°C respectively, in a fume cupboard.

The sediment samples were air dried, grinded using mortar and pestle and sieved through 2mm mesh size to remove coarse materials Then 5g of each of the 2mm mesh size sieved sediment samples were digested separately using (Aqua regia) 25ml of ratio 1:3 of concentrated Trioxonitrate (v) acid (nitric) (HNO_3) and Hydrochloric acid (HCl) at 105°C respectively, in a fume cupboard.

The fish samples were allowed to defrost. Then 2g of each of the fish samples tissue were digested separately using 25ml of ratio 1:1 of concentrated Nitric acid (HNO_3) and Hydrogen peroxide acid (H_2O_2) at 105°C respectively, in a fume cupboard (FAO/SIDA,1983).

2.4 Bio- concentration Factor in fish:

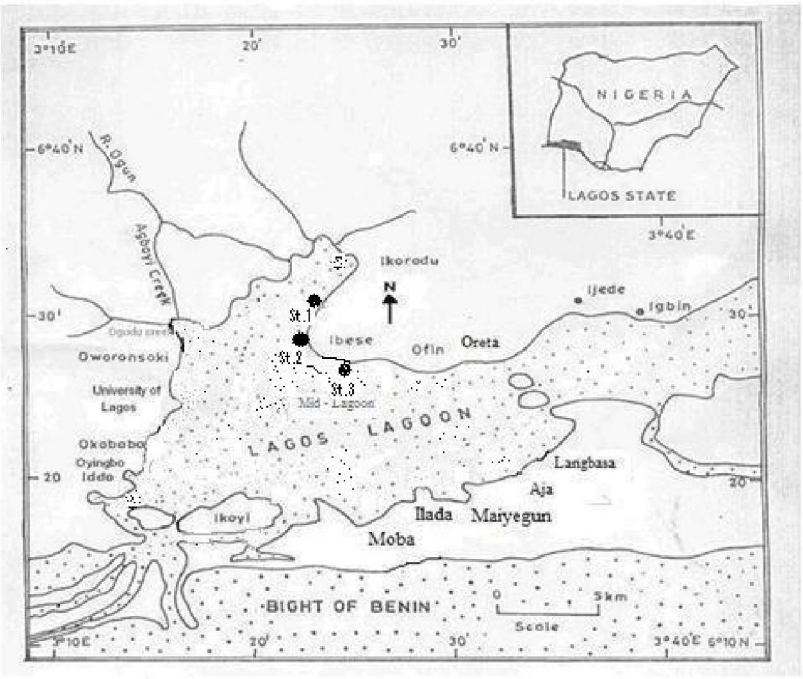
Bio concentration Factor (BCF) was determined in this study by calculating the ratio of the concentration of the metals in fish tissue to the concentration in the water (Walker et al, 1998).

3.0 RESULTS

The average of the physiochemical parameters of Ibeshe (Ikorodu) Lagos Lagoon are presented in Table 1. pH values of Ibeshe surface water ranges between 8.9 – 9.0 throughout the sampling periods. Conductivity was between 0.24 – 0.54 mS/cm. Turbidity values of between 24.8 - 156NTU. Freshwater salinity of 0‰, High Dissolved Oxygen content ranges between 4.20 – 7.80 mg/l, Alkalinity values of 10.0 – 16.0 mg/l and Water temperature ranged of 27 – 30^{0}C.

3.1 Heavy metals in water samples

The mean concentration values of studied heavy metals in water samples are presented in Table 2. The highest mean concentration value of Fe (0.330mg/l), Pb (0.120 mg/l) and Ni (0.250 mg/l) were obtained at station III. Station I and II gave the highest mean value of Cu (0.230 mg/l) and Cr (0.220 mg/l) respectively. Furthermore, highest mean concentration value of Zn (0.190 mg/l) was observed at both station I and II. In the case of Mn, station I and III recorded the highest mean concentration value of (0.120 mg/l). Consequently, the lowest mean concentration values of Fe (0.252 mg/l), Cu (0.120 mg/l) and Zn (0.148 mg/l) were obtained in the water samples from station II. Station III and I recorded the least mean concentration values of Mn (0.110 mg/l) and Ni (0.220 mg/l) respectively. Arsenic (As) was not detected in the three studied stations while Cd was below detection limits.



Map of study area showing the sampling sites ●

Table 1: The average physiochemical parameters of Ibeshe (Ikorodu) Lagos Lagoon

Parameters	pH	Conductivity (mS/cm)	Turbidity (NTU)	Salinity (‰)	DO(mg/l)	Alkalinity (mg/l)	Temperature (°C)
Range	8.9-9.0	0.24-0.54	24.8-156	0	4.2-7.8	10.0- 16.0	27-30
Mean	8.96	0.37	103.0	0	7.26	12.0	28

Table 2: The mean concentration (mg/l) of heavy metals in water of Ibeshe (Ikorodu) Lagos Lagoon at the three sampling stations (n = 5). (Range values in parentheses)
AMCS- Average means concentration of the three sampling stations
Means with the same superscript in each row are not significantly different ($p<0.05$).

Metals	Station I	Station II	Station III	AMCS	WHO/FEPA limits
Fe	0.300±0.026b (0.27-0.34)	0.252±0.011a (0.24-0.27)	0.330±0.024b (0.29-0.35)	0.293	**0.300**
Cu	0.230±0.017a (0.21-0.26)	0.120±0.012a (0.11-0.14)	0.180±0.010a (0.17-0.19)	0.177	**1.000**
Pb	0.100±0.017b (0.08-0.13)	0.100±0.007a (0.09-0.11)	0.120±0.007a (0.11-0.13)	0.107	**0.010**
Cd	ND	ND	ND	-	**0.003**
Zn	0.190±0.011a (0.18-0.21)	0.148±0.019b (0.13-0.18)	0.190±0.020b (0.17-0.22)	0.177	**3.000**
Cr	0.210±0.006a (0.20-0.22)	0.220±0.007a (0.21-0.23)	0.210±0.014b (0.19-0.23)	0.213	**2.000**
As	ND	ND	ND	-	**0.010**
Mn	0.120±0.006a (0.11-0.13)	0.120±0.010a (0.11-0.14)	0.110±0.012a (0.09-0.12)	0.117	**0.050**
Ni	0.220±0.017[a] (0.19-0.24)	0.230±0.002[a] (0.19-0.25)	0.250±0.022[b] (0.22-0.28)	0.233	**0.02**

3.2 Heavy metals in sediment samples

The mean concentration values of studied heavy metals in sediment samples are presented in Table 4. A cursory look at the Table 4 shown that the highest mean concentration values of Fe (93825.0µglg), Cu (62.75µglg), Pb (43.40µglg), Zn (122.80µglg), Cr (102.70µglg), As (1.10µglg), Mn (304.95µglg) and Ni (72.80µglg) were recorded in station III. Station I gave the highest mean value of Cd (1.08µglg). Meanwhile, the least mean concentration values of all the studied metals Fe (78371.0µglg), Cu (44.57µglg), Pb (34.95µglg), Cd (0.90µglg), Zn (94.95µglg), Cr (82.70µglg), As (0.90µglg) and Mn (256.30µglg) in sediment samples were recorded in station II except the least mean concentration value of Ni(63.85µglg) which was observed at station I.

Table 3: The mean concentration (µg/g) of heavy metals in sediment of Ibeshe (Ikorodu) Lagos Lagoon at the three sampling stations (n = 5). (Range values in parentheses).
AMCS- Average means concentration of the three sampling stations
Means with the same superscript in each row are not significantly different ($p<0.05$).

Metals	Station I	Station II	Station III	AMCS	Unpolluted Sediments (values) GESAMP, 1982.
Fe	83715.0±76.47a (83615-83830)	78371.0±325.27b (77870-78770)	93825.0±1838.5c (91225-96425)	85303.33	**41000**
Cu	52.90±1.21a (51.35-53.95)	44.57±3.00c (41.20-47.70)	62.75±1.84a (60.05-65.10)	53.967	**33**
Pb	39.80±21.51c (17.95-75.45)	34.95±16.91b (10.35-57.35)	43.40±4.39a (36.35-48.10)	38.350	**19**
Cd	1.080±0.08a (1.00-1.20)	0.90±0.08a (0.80-1.00)	1.00±0.19a (0.75-1.25)	1.000	**0.11**
Zn	112.80±7.57a (101.50-122.70)	94.95±12.67b (77.80-113.50)	122.80±21.78c (91.95-152.70)	110.183	**95**
Cr	96.25±17.11a (72.90-113.60)	82.70±34.30b (44.75-122.80)	102.70±54.60c (46.20-172.90)	93.880	
As	1.05±0.40b (0.50-1.55)	0.90±0.20a (0.60-1.10)	1.10±0.42b (0.60-1.70)	1.017	**14**
Mn	283.90±92.98b (176.40-421.70)	256.30±95.41b (117.70-342.60)	304.95±57.06a (221.20-356.10)	274.967	**770**
Ni	63.85±8.11b (56.72.60)	65.55±17.49c (48.75-94.95)	72.80±2.51a (70.25-76.10)	67.40	

3.3 Heavy metals in fish (*Chrysichthys nigrodigitatus*) samples

The mean concentration values of heavy metals in the fish tissue are presented in Table 5. The highest mean concentration value of Fe (4.313µglg) was recorded in fish samples collected at both station I and III while the lowest mean value (4.163µglg) was obtained in fish samples caught at station II. The highest mean concentration values of Cu (10.663µglg), Zn (15.713µglg) and Cr (1.663µglg) were observed in station III while the least Cu (5.975µglg), Zn (8.350µglg) and Cr (1.100µglg) were recorded at station II. Highest mean concentration values of Ni (5.725µglg) and Pb (2.225µglg) were obtained at station I while the lowest mean values of (1.650µglg) and (1.125µglg) were from the fish samples at station II. Meanwhile, fish samples from station II recorded the highest mean concentration values of Mn (1.730µglg) and Cd (1.375µglg) while the lowest Mn mean value of (1.225µglg) was obtained from fish samples caught at station II. Cd was below detection limit at station II and III while Arsenic was not detected in all the fish samples collected from all the sampling stations.

3.4 Bio-concentration factor

The mean bio-concentration factor (BCF) of heavy metals was as presented in Figure 2. Generally a relatively high BCF values were observed in this study. The highest BCF of Fe (16.5) was recorded in fish samples obtained at station II followed by 14.4 and 13.1 from stations I and III respectively. Station III fish presented the highest value for Cu (59.2) followed by stations II and I respectively. Fish samples from station II also have the highest Mn (14.4) while station I fish samples have the highest

BCF values of Pb (22.3) and Ni (26.0).The highest BCF values for Cr(7.9)and Zn (82.7) were observed at station III. The BCF values for Cd and As (Arsenic) could not be determined.

Table 4: The mean concentration (µg/g) of heavy metals in fish (*Chrysichthys nigrodigitatus*) tissues of Ibeshe(Ikorodu) Lagos Lagoon at the three sampling stations (n = 5). (Range values in parentheses).
AMCS- Average means concentration of the three sampling stations
Means with the same superscript in each row are not significantly different (p<0.05).

Metals	Station I	Station II	Station III	AMCS	WHO limits
Fe	4.313±0.953b (2.950-11.050)	4.163±0.841a (2.938-5.125)	4.313±1.204c (2.625-5.750)	4.263	**800.00**
Cu	8.050±3.137c (3.888-11.050)	5.975±1.962b (3.900-8.713)	10.663±1.391a (8.925-12.760)	8.229	**30.00**
Pb	2.225±0.463a (1.563-2.763)	1.558±0.613b (1.125-2.625)	2.113±1.353c (0.563-4.025)	1.967	**2.00**
Cd	ND	1.375±1.76a (0.25-4.38)	ND	0.458	**2.00**
Zn	9.988±1.326a (8.350-11.760)	8.350±1.606b (6.375-10.730)	15.713±1.886c (13.090-16.910)	11.338	**1000.00**
Cr	1.225±0.339a (0.638-1.500)	1.100±0.916b (0.263-2.550)	1.663±1.146c (0.138-2.513)	1.329	**150.00**
As	ND	ND	ND	ND	**3.00**
Mn	1.663±0.689c (0.813-1.888)	1.730±0.518b (1.350-2.625)	1.225±0.440a (0.563-1.688)	1.513	–
Ni	5.725±1.967c (3.513-8.300)	3.188±1.174a (1.650-4.738)	3.225±1.566b (1.313-5.438)	4.046	–

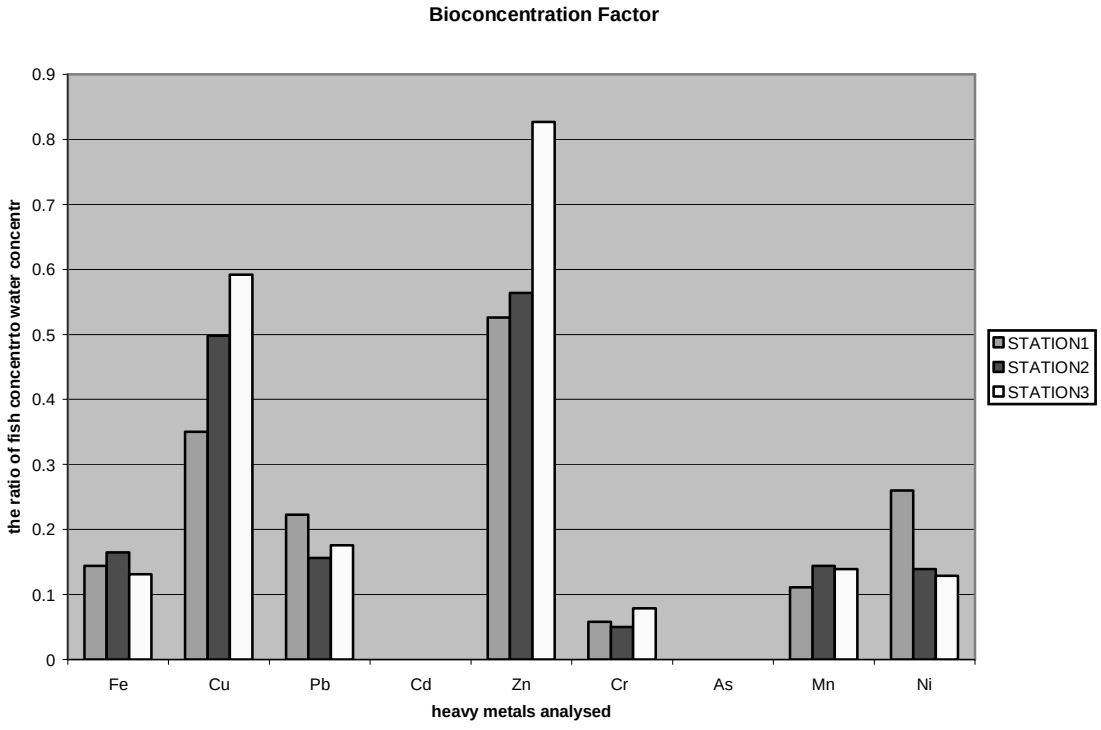


Figure 5: Bio-concentration factor (BCF) of heavy metals in fish of Ibeshe (Ikorodu) Lagos Lagoon

4.0 Discussion

Water temperatures were fairly constant throughout the study period. These temperatures have been reported by several authors in the Lagos Lagoon (Nwonkji et. al, 2010, Ajao, 1989). pH observed in Ibeshe throughout the sampling periods was alkaline. Similar findings have been reported (Nwankwo, 1996). Buffer properties of the water were responsible for this stable pH. Conductivity and Salinity have been established as associated factors (Onyema and Nwankwo 2009). Salinity recorded throughout in this study typifies freshwater condition and decrease with decline in Conductivity. High turbidity reported could be attributed to release of particulate matter brought in by rain. High Dissolved Oxygen recorded could be linked to mixing due to rainfall.

All heavy metals analyzed in this study were detected in water, sediment and fish samples (*Chrysichthys nigrodigitatus*) of Ibeshe (Ikorodu) Lagos lagoon except Cd and As that were below detection limit in all the study media (water, sediment & fish) from all the study stations.

Metals concentrations in water at all three sampling stations were below their concentration in the sediment (Table 2&3). This is similar to the findings of Amoo et al., 2005 and Sabo et al.,2008 where high concentration of metals were observed in sediment of Lake Kainji and River Gongola when compared with their surface water. The reason for this may not be unconnected with the fact that pollutant discharge into aquatic environment does not remain in aqueous phase but instead they adsorbed onto the sediment, since sediment serves as a sink for pollutants. The metals adsorbed to sediment are however remobilized back into surface water through changes in some physiochemical parameters such as temperature, pH and redox potential. The average physiochemical parameters of the study areas such as pH, Alkalinity and surface water temperature observed (Table 1) in the course of this study favour adsorption of the metals in the sediment rather than remobilization.

The mean value of Cu, Zn and Cr in water samples from the three sampling stations are below the FEPA/WHO threshold limits of 1.000mg/l, 3.000mg/l and 2.000mg/lrespectively while Pb, Mn and Ni in the water samples from the three sampling stations are above the WHO/FEPA limits of 0.01mg/l, 0.05mg/l and 0.02mg/l respectively. Fe in water samples from stations I & II are within the acceptable limit of FEPA/WHO of 0.300mg/l while the Fe mean value in water samples in station III is slightly above the threshold limit of FEPA/WHO.

The mean value of all the metals in the sediment collected at the three sampling stations exceed the GESAMP, 1982 and Solomon & Forstner, 1984 limits for unpolluted sediment except As and Mn that are below the recommended limits of 14µg/g and 770µg/g for unpolluted sediment. It is discernable from Table 4 that sediment of Ibeshe (Ikorodu) Lagos lagoon is highly polluted with all the studied heavy metals except As and Mn.

A critical look at the mean values of metal content in fish samples from all the three sampling stations (Table 4) revealed that all the studied metals are below the WHO threshold limits except Pb in station I & II that are above the threshold limits of WHO. The level of Pb observed in the studied fish poses no risk to public health but required constant monitoring.

The BCF determined for different metals revealed that bioaccumulation has occurred in the fish but not in alarming rate. This may be attributed to fact that *Crysichthys nigrodigitatus* is not a benthic fish that always have constant contact with the sediment and the average physiochemical parameters of Ibeshe; Lagos lagoon (Table 1) observed to be favourable to the adsorption of these (Fe, Cu, Pb, Cd, Zn, Cr, As, Mn and Ni) metals on to sediment rather than their remobilization. These reduce the level of heavy metals in surface water that are available for bioaccumulation in fish.

Conclusion

Although heavy metals in aquatic environment are from natural sources (soil formation), non point sources, transportation, domestic wastes, urban run-off, Agricultural practices and industries. Industrial activities such as industrial effluent discharge have been reported as a major contributing factor (Ademoroti and Sridhar, 1979, Abdel-Shafy and Abdel-Bashir, 1991, DeGregori *et al*., 1996, Agarwark, 1999 and Asia and Ademoroti, 2001).

Therefore, the appreciable level of heavy metals observed in the water, sediment, and fish (*Chrysichthys nigrodigitatus*) of Ibeshe (Ikorodu) Lagos lagoon has revealed the impact of the industrial effluent discharged on the Ibeshe in Lagos lagoon. This is a thing of concern as the presence of the following sets of metals (Pb, Mn & Ni), (Fe, Cu, Pb, Cu, Cd, Cr & Zn) & (Pb) were above their threshold limits in the studied water, sediment and fish respectively. Consequently, there is need for **NESTRA** and **Lagos Ministry of Environment** to embark on continual assessment of the effluent discharge of the industries situated at Ibeshe and other activities that could be injurious to human

health and the ecological integrity of the Ibeshe Lagos lagoon ecosystem.

The results reported in this study would also be expected to serve as baseline level for future heavy metal pollution status of the Ibeshe, Lagos Lagoon area.

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06/07/10

Breeding success of Lesser Crested Tern and Swift Tern at Shidvar island, Iran

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ABSTRACT: The aim of this study was to investigate the breeding success of Lesser Crested Tern *Sterna bengalensis* and Greater Crested Tern *Sterna bergii* at Shidvar Island, in Persian Gulf, southern of Iran. Total Count Method that included tree breeding colonies was carried out. A total of 365 nests, belonging to 240 nest of Lesser Crested Tern and 125 nest of Swift Tern, were categorized under number of eggs and were counted. The mean clutch sizes of Lesser Crested Tern and Swift Tern were estimated 1.04±0.01 and 1.04±0.03 respectively. Furthermore, the average of breeding success during incubation of eggs, nestling and post-nestling were measured 67.7%, 100% and 95.24% for Lesser Crested Tern and 83.3%, 70% and 100% for Swift Tern. The total breeding success was measured 74.43% and 66.63% for them respectively. Relative abundance of birds during outward migration was measured 65.32% and 34.68% for two species, respectively. It is considered that the importance of Shidvar Island for seabirds, especially for family of Sternidae, must be recognized and the protection of this site from threats must be enforced.

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KEYWORDS: Breeding Biology, *Sterna bengalensis*, *Sterna bergii*, Shidvar, Iran

1. INTRODUCTION

The breeding areas in the islands are known as a most important biological area for Tern spp. (Burger & Lesser, 2008), and are detected as indicators for dynamic process of their population's control (Elliott *et al*., 2007). Moreover, results of tern communities study also reliable as a tool to monitoring of habitat (Arnold et al., 1998).

The species of Lesser Crested Tern *Sterna bengalensis* and Greater Crested Tern *Sterna bergii* are a very common seabird along the entire south coast from the region of Khark Island in Bushehr province to the Pakistan border in Baluchestan province (Scott, 2007). They are under the species to which the *Agreement on the Conservation of African-Eurasian Migratory Waterbirds* (AEWA) applies (Azafzaf *et al*., 2006). However, Shidvar hosts the most important colony of Lesser Crested Tern *Sterna bengalensis*, Greater Crested Tern *Sterna bergii*, Bridled Tern *Sterna anaethetus* and White Cheeked Tern *Sterna repressa* in the Persian Gulf in Iran, there has been little discussion about breeding biology and phenology of this marine birds in this area. Likewise, there have been no controlled studies which compare differences in suitability of island to breeding success of terns. Despite its special importance, regular scientific studies have not been conducted on Shidvar Island and related subjects and most of the confirmed reports are outdated. This is a cause for concern. Furthermore, research of DoE have focused on birds counting during limited time, however, there has been an increasing interest in ringing of terns in recent years. This study presents detailed overviews of the breeding success of Lesser Crested Tern and Swift Tern at the Shidvar Island, Iran

2. MATERIAL AND METHODS

2.1. Study Area

The Shidvar Island, with 98 ha area, is located at 9 km offshore in the Hormozgan province, East of

Lavan island, in the Persian Gulf, southern of Iran, within 26˚47′–26˚48′N and 53˚24′–53˚25′E. It was designated as a wildlife refuge in 1971. In addition, due to its importance as a nesting site for migratory bird species (especially *Sterna* spp.), the island was nominated in 2000 as Iran's 20th international wetland in Ramsar sites.

Only the eastern and northern beaches of the island are sandy. The eastern part of the island is smooth and flat, but the northern part is composed of both gradient and less gradient beaches and is rocky in some places. Considering the limiting factors on Shidvar, such as low rainfall and the soil, which is completely sandy, there are a few species of plants on the island. The vegetation in the eastern and central areas is dense; include halophyte species such *as* Sueda spp., Atriplex sp. and *Cyperus rotundus*.

Except of terns, birds such as *Egretta gularis, Arenaria interpres* , *Galerida cristata, Columba livia, Phalacrocorax nigrogularis*, *Larus hemprichii*, *Prinia gracilis*, *Numenius arquata*, *Pandion haliaetus*, *Corvus ruficollis*, *Charadrius leschenaultia*, and reptiles such as *Echis carinatus sochureki*, *Scincus scincus conirostris*, *Mabuya aurata septemtaeniata*, *Mesalina watsonana*, *Eretmochelys imbricata bissa* and *Chelonia mydas japonica* were recorded during period of study in Shidvar Island or locally name 'Marou', which means island with too many snakes.

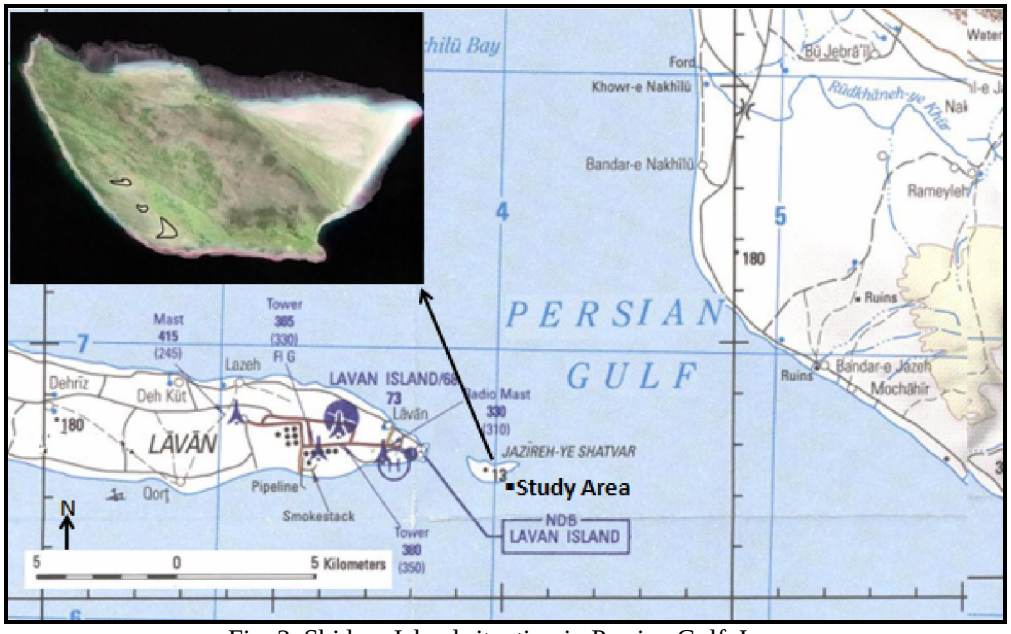

Fig. 2. Shidvar Island situation in Persian Gulf, Iran

2.2 Methods

The study was conducted with data being gathered via daily direct observation from 1st April to 1st September 2007. Total Count Method was carried out in three colonies of breeding. Monitoring of sampling site was carried out during 2008 and 2009. The survey was conducted from 0800 hrs to 1400 hrs. A pair of binoculars (10x) and spotting scopes (30x and 60x) were used for detecting and identifying birds.

The characteristics of eggs, chick, juvenile and adults such as size, form, painting, flight, song and Bird classification were identified by examining every nest and using the bird's field guide books (Mansoori, 2001; Baicich & Harrison, 2005; and Harrison & Castell, 1998). The characteristics of eggs (e.g. diameter, length and width) were measured using caliper with precision 0.01 cm and weight was measured using digital scales. The semi-structured approach was chosen by Holloway (1993); Spendelow & Zingo (1997); Elliott *et al.* (2007); and Behrouzi-Rad & Hosseini-Tayfeh (2008).

2.3 Data Analysis

The Eggs Volume (EV) (cm^3) and Eggs Form Index (EFI) were determined using expressions: *EV (cm^3) =K x L (m) x (B^2) (cm)* and *EFI=B/L x 100* respectively (Where *K* is fixed number, usually equal to 0.4866, *L* and *B* are length and width of eggs). The relative abundance (%) was determined using expression: *n/N x 100* (Where *n* is numbers of recorded and *N* is total observations recorded). All values were represented as Mean ± Standard error of the mean (SE). Data management and analysis were performed using SPSS 16.0 and Excel 2007. Degree of threatened was founded based on IUCN reports (2008) in http://www.iucnredlist.org/.

3. RESULTS

The population and breeding biology of Lesser Crested Tern *Sterna bengalensis* and Swift Tern *Sterna bergii* were studied at Shidvar islands. Several reproductive parameters have been recorded during 2 years (from 2007 to 2008), such as clutch size, egg size and breeding success, as well as the number of breeders during the study and their population dynamics.

The result of observations showed that the first arrival date of terns to Shidvar Island occurs on April 26th till May 2nd. Terns breed collectively in the three separate small colonies and in association with together during their breeding period, which begins at the second decade in May (9-14th).

Nests were built in open areas on the small rocky cliff and free of vegetation in the southwest of island, with 200 meters distance from the beach.

A total of 730 flying and alarm calling birds in the 365 nests, belonging to 240 nest of Lesser Crested Tern and 125 nest of Swift Tern, were recorded, categorized under species and number of eggs and were counted (Table 1). Directly observations illustrated that although breeding of these species were highly synchronous, females laying first in each species were probably in best body condition. In addition, it showed that laying of swift tern was earlier. Laying were also, observed in June and seems that period of laying is about one month, and also the male offers fish to the female as part of the courtship ritual.

Out of the total number, one or two eggs were laid by these monogamous birds, and incubated by both parents to hatching, which 95.4% nests of Lesser Crested Tern were recorded with 1 egg, while it was 99.2% for swift tern nests. The average clutch size was estimated 1.04±0.01 and 1.04±0.02 respectively (Table1). The eggs were differing mainly in the color, olive yellow to pink and cream peas yellow to white with blackish streaks.

Table 1. Clutch Size of Terns in Different Colonies, Shidvar Island (2007)

No. Colony	Lesser Crested Tern			Swift Tern		
	No. Nests	No. Eggs	Ave. Clutch Size	No. Nests	No. Eggs	Ave. Clutch Size
1	103	97	1.06	56	56	1.00
2	51	49	1.04	18	19	1.06
3	86	83	1.03	51	51	1.00
Mean	80	83.67	1.04	41.67	42	1.02
SE	15.31	16.39	0.01	11.92	11.59	0.02

The mean of length (mm), width (mm) and weight (g) of eggs were measured 49.33±0.53, 33.93±0.38, 32.33±0.79 for Lesser Crested Tern and 59±0.66, 46.27±0.54, 59.6±0.8 for Swift Tern, respectively. Also, The Eggs Volume (EV) and Eggs Form Index (EFI) were measured 27.78±0.91 cm^3 and 68.81±0.44 for Lesser Crested Tern and 61.75±2.03 cm^3 and 78.44±0.56 for Swift Tern respectively.

The average breeding success during incubation of eggs, nestling and post-nestling were measured 67.7%, 100% and 95.24% for Lesser Crested Tern and 83.3%, 70% and 100% for Swift Tern. The total breeding success was measured 74.43% and 66.63% for them respectively. However, the young terns fledge after 38 to 40 days; remain dependent on the parents after leaving the colony until they are about four months old. Furthermore, duration (days) of Reproductive Stage include Nest Building, Egg laying, Incubation, Nestling, Post-nestling, and Flying

were measured 1.06±0.31, 3.52±0.28, 22.03±1.41, 3.19±0.22, 19.41±1.54, and 2.2±0.1 for Lesser Crested Tern and 3.24±0.62, 2.31±0.17, 27.03±1.52, 6.35±0.76, 28.7±1.73, and 4.3±0.4for Swift Tern respectively (Table 2). The result of observations showed that the first arrival date, first laying date and hatching out of Lesser Crested Tern were April 26th , May 14th , and June 8th respectively. While, these date were April 26th , May 9th, and June 7th for Swift Terns at Shidvar island, respectively. The results of observations indicated that total stages extend till end of August for both of terns (Table 3).

The population size of swift tern in the first and end of migration and breeding periods at Shidvar Island were measured 480 and 648 individuals for Lesser Crested Tern, while there were 250 and 344 individuals for Swift Tern, respectively. Therefore, relative abundance (%) of birds during inward and outward migration were measured 65.75% and 65.32% for Lesser Crested Tern, while there was 34.25% and 34.68% for Swift Tern, respectively.

Table 2. Duration (days) of Reproductive Stage, Shidvar Island (Numbers are range of variation)

Species	Nest Building	Egg laying	Incubation	Nestling	Post-nestling	Flying
Lesser Crested Tern	1.06±0.31 (1-4)	3.52±0.28 (2-4)	22.03±1.41 (20-24)	3.19±0.22 (2-4)	19.41±1.54 (20-25)	2.2±0.1 (1-4)
Swift Tern	3.24±0.62 (3-5)	2.31±0.17 (2-4)	27.03±1.52 (25-28)	6.35±0.76 (6-10)	28.7±1.73 (25-32)	4.3±0.4 (1-7)

Table 3 Starting Date in terms of Terns Reproductive Phenology, Shidvar Island (2007)

Species	First Arrival	Laying			Hatching Out	Nest Leaving	First Flying	Outward Migrations
		First	Peak	End				
Lesser crested T.	26April	14May	14-19May	2june	8June	13 June	Mid-Aug.	Late Aug.
Swift T.	26April	9May	9-14May	2June	7June			

4. DISCUSSION

More recently, literature has emerged that offers contradictory findings about biology of Terns (Arnold *et al*., 1998; Burger & Lesser, 2008; Elliott *et al*., 2007; Eyler *et al*., 1999; Perrins, 2008; Wright *et al*., 2010). However, far too little attention has been paid to the Hormozgan province in Iran. However, DoE is counting the wintering or summering bird communities every year, but there have been no controlled studies which monitor population of Sternidae and their breeding biology. In this investigation, the aim was to assess breeding success of Lesser Crested Tern and Greater Crested Tern (Swift Tern) in the Shidvar Island.

Groups of Terns on the island of Shidvar have been recorded in the past. Scott (2007) reported breeding of Lesser Crested Tern (1000 adults and 10 nests in 1972, and 40 adults and 10 nests in 1977), and Swift Tern (30–40 adults and 1 nest in 1972; 100 adults and 4 nests in 1977) on Shidvar Island. In this study, a total of 365 breeding pairs in 2007 were identified that increased slightly to 382 in 2009 (mean annual growth rate was 1.05 per year).

The total number of breeding pairs increased slightly from 365 pair in 2007 to 382 in 2009 (mean annual growth rate was 1.05 per year). The most of the breeding parameters analyzed showed there are no statistically significant differences between years. Although the rate fluctuated greatly between years, these changes were probably related to many factors such as habitat condition and also human disturbances effect.

The present findings seem to be consistent with other research which found these terns feed by plunge diving or dipping, usually in the top 1 m of water (Hockey *et al*., 2005) and only fish near the surface are available as food (Crawford, 2009). This finding is in agreement with Crawford (2003) findings which showed numbers of breeding population were significantly related to food availability and biomass of small fish such as Clupeidae (e.g. sardine). Another important finding was that the colony size is related to the abundance of pelagic fish prey and distance of colonies from the beach. In addition, this study suggested that in general good recruitment to the mature population may have contributed to the increase.

The study of breeding access illustrated that area has a good stability in environmental condition, availability of food, nest sites and predation rates for a varieties of seabirds such as terns. However, this area is

under DoE Wildlife Refuges List and is under Ramsar Sites, this is consider that area is a sensitive and brittle habitat for breeding species such as terns, it is need to protect better than this, especially during breeding time of Terns and Turtles.

This study indicated that although no one lives on Shidvar Island, the adjacent island- Lavan, with an area of about 76 sq km- is notable because of its refinery, gas and oil exploitation and transportation infrastructure. Apart from the related staff, some local people are living there as well. These people are engaged in fishing and sea transportation activities. Therefore, main threats for these birds are human disturbance, fisheries, egging, gas and oil exploitation, which all of them related to human activity. Another main threat of breeding is natural predator of tern eggs such as Ruddy Turnstone *Arenaria interpres*, and eastern saw-scaled viper *Echis carinatus sochureki*, during laying and nestling time. It has often been recorded feeding on eggs and early chicks. Behrouzi-Rad & Hosseini-Tayfeh (2008) also recorded feeding of eggs by turnstone and Crab Plover. On the other hands, lack of counts at some coastal sectors and changes among breeding sites between seasons preclude an accurate estimation of total population size for both species and make spatial management challenging.

However, this study showed terns bred at a number of other suitable offshore islands for breeding in Hormozgan province (e.g. Siri, Tonb, Abu-moosa), which were never surveyed, and adjacent province such as Bushehr's islands (e.g. Khan, Tahmadon, Nakhiloo and Um-al-Gorm) (Behrouzi-Rad & Hosseini-Tayfeh, 2008), it found that Shidvar holds the largest Tern's colony in Persian Gulf limit in Iran (a total of 250000 pairs belonging 4 species). Therefore, it is considered that the importance of Shidvar island for seabirds, especially for family of Sternidae, must be recognized and the protection of this site from threats must be enforced.

It is recommended that further research be undertaken in the following areas: To regularly monitor the two known breeding colonies at all suitable islands of province; To ring all young at the colonies in order to get a better idea of the movements; To monitor the breeding avifauna of Shidvar and to clarify its importance and habitat changes for conservational activities.

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Transmissivity of the Glazing Surface of a Solar Flat Plate Collector Based on the Metrological Parameters of Yola, Nigeria

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Abstract: A glazing surface is one of the most vital components of a solar flat plate collector which is meant to admit maximum possible radiation and minimizes upward loss of heat. The most commonly used glazing surface is transparent glass. The performance of the glazing surface depends on the magnitude of its transmissivity. For a given material, this optical property is a function of solar geometry that varies with geographic location. In this work, the monthly mean value of transmissivity of the most commonly used glazing surface, 3mm transparent glass was determined for 12 months of the year with respect to the solar geometry of Yola town. A peak value of 0.8823 was recorded in the month of September while a minimum value of 0.8775 was recorded in the month of January. An annual mean value of 0.8807 was recorded with a standard deviation of 0.0015. The results imply that plane glass as a glazing surface admits about 88% of the solar radiation incident on it to the absorbing surface. The slight variation all year round is an indication of its consistent performance all times of the year at the locality.
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Key words: transmissivity, plane glass, glazing cover, flat plate collector, solar energy

1. Introduction

The current trends in global energy demand and the environmental impact of excessive exploitation of conventional energy sources necessitate the need for diversification of energy sources. Owing to the ever growing global population and economic growth of developing countries, the global energy demand increases at the rate of 1.2% per year and it is projected to be 35% more that the current demand by the year 2030 (Exxolmobil, 2010). This comes at a time when about 90% of the world's primary energy supply comes from the non renewable fossil fuel (International Energy Agency, 2010). The resources are therefore fast deflating due to excessive exploitation in an attempt to meet the ever increasing demand. Consequently the price continuously rise over the years thereby causing hardship in addition to increased greenhouse gas emission which leads to an irreversible damage to natural habitat.

Significant population of many developing countries of Asia, Africa and the Caribbean depends on the fossil fuel as a conventional energy sources for domestic use. In Nigeria, the conventional sources of energy for domestic cooking like liquefied petroleum gas (natural gas), kerosene and electricity are characterized by irregular availability, increased in cost and mostly not environmentally friendly (Bello, Makinde & Sulu, 2010). This necessitates the need for intensive effort toward harnessing non renewable and environmentally friendly energy sources.

The applicability and popularity of solar technology is therefore widening in Nigeria as it is in both developed and developing countries due to its simplicity and environmental friendliness as people are battling with the challenge of meeting their energy demands. The effective performance of any solar system however requires favourable weather conditions and good insolation. Tropical regions of the world have greater potential application of solar technology due to their relatively large solar insolation, minimal variation of daily sunshine hours and large sky clearness index (Mzad, 2008).

The town of Yola, the capital of Adamawa state in North-Eastern Nigeria, lies within Latitude: $09^{o}.14'$N and Longitude: $012^{o}.28'$E at a mean altitude of 174 m above sea level within the upper Benue trough of North-Eastern Nigeria. The town receives abundant daily sunshine and has maximum annual ambient temperature ranging between 32^{0}C to 43^{0}C (Adebayo and Tukur, 1999). Thus the town lies within a high sunshine belt of the country and solar radiation across it is fairly distributed. According to metrological data, the town receives an average of about 2.18 $X10^{7}Jm^{-2}$

per day from the sun; equivalent to 774.84Wm^2. This is significantly higher than the country's annual mean of about 1.89X10^{7}Jm^{-2}-day (Sambo and Taylor, 1990). The intensity however varies fairly with time from about 1.58X10^{7}J/m^2-day in August to about 2.58X10^{7}J/m^2-day in the month of March. The current electricity demand of the entire Adamawa state is about 4000MW. This could have been provided with this mean intensity of 774.84Wm^2 falling in an area of 0.4km^2 (40 hectares) of land if a conversion device of only 10% efficiency is use. This enormous potentiality raises the need for intense research in the field with a view of maximizing its benefit.

One of the most effective methods of converting solar energy to useful form is heliothermal, the process of converting solar radiation to useful thermal energy. It is the principle of operation of many solar devices such as solar cooker, solar still, solar water disinfection, solar power desalination etc. The most important component of heliothermal system is the flat plate collector. Both liquid and gas flat plate collectors as well as photovoltaic cell consist among other things, a glazing which may be one or more sheets of glass or any other radiation transmission material. Glass is the most commonly used material as it can transmit up to about 90% of the incident short wave radiation while its transmittance to the long wave heat radiation (5 to 50 μm) emitted by the absorber plate is very negligible (Tiwari 2002). In this work, the effectiveness of 3mm transparent glass as a glazing surface is tested with respect to the metrological parameters of Yola town. The aim of this work is to determine the transmissivity for the most commonly used glazing surfaces: 3mm transparent glass. This is with a view of assessing its performance under the solar geometrical condition of Yola town. The work therefore provides a useful data in analysing the thermal performance of a prototype solar collector. It also serves as an appraisal of the impact of geographic location and seasonal variation of metrological parameters to the performance of glass as a glazing cover.

2. Parametric definition and computation

The intensity of solar radiation striking the absorber plate of a flat plate collector depends on the transmission properties of the glazing cover. The rate at which energy is absorbed by a plate per unit area $\dot{q}_{ab}$ is related to the solar intensity $I(t)$ by the equation (Tiwari , 2002)

$$\dot{q}_{ab} = (\tau\alpha)I(t) \qquad 1$$

where τ is the transmittance or transmissivity of the glazing cover. Transmissivity of the cover is therefore very important in designing and evaluating the performance of solar energy conversion systems. It varies with geographic location due to the variation of solar geometry with location (Sukhatme 1984). The parameter depends on solar geometry and can be obtained using the following solar geometrical factors:

Declination δ **.** This is the angle between the lines joining the centres of the sun and the earth with its projection on the equatorial plane. It is given by Cooper's equation (Mzad, 2008) as

$$\delta = 23.45\sin\left[\frac{360}{365}(284+n)\right] \qquad 2$$

where *n* is the day of the year. For this work, n is taken as the last day of each month.

Hour angle ω . Is the angular measure of time, equivalent to 15^0 per hour. It is measured in the noon based on local apparent time (LAT).

Local Apparent Time (LAT) is defined by Sukhatme (1984) as

LAT = Standard time $\mp$ *(standard time longitude) - (longitude of location) + (equation of time correction).* 3

The negative sign in the first term is applicable to eastern hemisphere while the positive sign is applicable to western hemisphere.

Slope β is the angle made by the collector plane surface and the horizontal (Fig. 1). The equation of time correction, which is a correction due to the fact that the earth's orbit and rate of rotation are subject to small perturbation, is based on experimental observation. A correction chart was given by Sukhatme (1984) from where correction for the last day of each month of the year, taken as the days of the experiment, was obtained (table 1).

In Nigeria, standard time is based on longitude 15^{o}E. For 13:00hours (1:00pm) of the experimental day at Yola (Long. 12^o 28'E), LAT is calculated for the 12 months of a year from the expression:

LAT=1300hrs-4(15^o-12^o.28')min + (equation of time correction for the month). 4

The incident angle of the beam radiation θ_i is given by Tiwari (2002) as

$$\sin\theta_i = \sin\delta\sin(\phi-\beta)+\cos\delta\cos\omega\cos(\phi-\beta)$$

where

ϕ is the latitude of the location. In this work, the collector is tilted at latitude angle. Thus $\phi = \beta$ and therefore

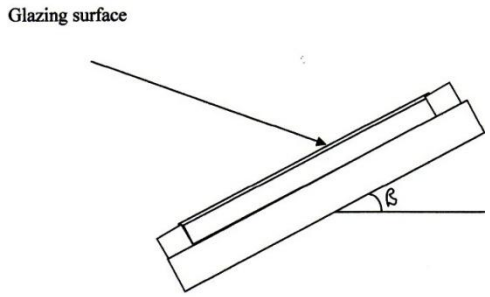

Fig. 1. Plane glass glazing cover

$$\sin\theta_i = \cos\delta\cos\omega. \qquad 5$$

The refracted angles are given by Snell's law as

$$\theta_r = \sin^{-1}\left[\frac{\sin\theta_i}{n}\right] \qquad 6$$

The reflectivities for both the beam and diffused components of radiation are respectively given by the equations (Tiwari, 2002)

$$\rho_1 = \frac{\sin^2(\theta_r - \theta_i)}{\sin^2(\theta_r - \theta_i)} \qquad 7a$$

$$\rho_2 = \frac{\tan^2(\theta_r - \theta_i)}{\tan^2(\theta_r + \theta_i)} \qquad 7b$$

The transmissivity based on the reflection τ_r is given by the equation (Garg and Prakash, 1997)

$$\tau_r = \frac{(\tau_{r1} + \tau_{r2})}{2} \qquad 8$$

Where

$$\tau_{r1} = \frac{1-\rho_1}{1+\rho_1} \quad \text{and}$$

$$\tau_{r2} = \frac{1-\rho_2}{1+\rho_2}$$

The transmissivity of the cover τ is given by

$$\tau = \tau_r\tau_o \qquad 9$$

Where τ_o is the transmissivity based on absorption given by Bouger law (Sukhatume, 1984) as

$$\tau_o = e^{-\frac{k\delta}{\cos\theta_r}} \qquad 10$$

k is the property of the glass cover known as its extinction coefficient. Its value for a plane glass varies from about 5 to 25m^{-1} depending on the glass quality. Assuming the glass cover is of average quality, *k*=15m^{-1}. The glass cover used for this work is plane of thickness δ =3mm. Thus τ_o and τ are computed for the 12 months as shown in table 1.

3 Result and discussion

The computed results based on the above definations and inputs are summarized in table 1. Fig. 2 is the plot of the variation of transmittvity τ with time of the year. With a mean value of 0.8807 and a standard deviation of just 0.0015, the variation over a year is insignificant. It however follows the trend of the variation of solar intensity with time. The results as illustrated in Fig. 2 reveals that monthly values of transmissivity fluctuates around an average value of 0.8807 each season. It is however noted that with a standard variation of just 0.0015, the variation is less significant. A peak value of 0.8823 is recorded in the month of September while a minimum value of 0.8775 is recorded in the month of January. The plot shows two peak regions within the months of February-March and August-October. The implication is that solar flat plate collectors constructed with this plane glass as a glazing cover performs at a relatively higher efficiency within these periods when compared to the minimum periods of November-January. It worth nothing however that with a mean value of 0.8807, about 88.07% of the solar intensity falling on a 3mm transparent glass glazing cover under the condition of Yola solar geometry is transferred and transmitted to the absorber plate. This amount is nearly constant with a slight variation over the year. The slight variation over the year is an indication of a year–round reliability and good consistency of a plate glass as a glazing cover in the locality

4. Conclusion

The efficient and effective performance of the glass glazing surface within the town is yet another indicator to the fact that Yola town is endowed with abundant solar energy potentials. Regrettably like the remaining part of the country however, the population almost entirely depends on the non renewable conventional energy sources mostly fossil fuels and biomass for domestic use. With an annual mean transmissivity value of 0.8807, the glazing surface is capable of all year round performance and therefore consistently reliable within the locality. It therefore provides an opportunity for the most efficient means of converting solar energy to thermal and electrical energies if used as glazing cover on a solar flat plate collector or photovoltaic cell. If placed in a convenient location say the roof of buildings, the devices will provide domestic heating, purification and electricity

for domestic use cost effectively and will therefore supplement the deflated conventional source.

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Appendix

Table .1, transmissivity computation table

Month	***n***	δ (°)	***Eqn of time corr'n (min)***	***LAT***	ω/°	θ_I	θ_r	ρ_1	ρ_2	τ_{r1}	τ_{r2}	τ_r	τ_0	τ	$(\tau\alpha)$
1	31	-17.78	-3	12.47	-7.05	19.08	12.59	0.0507	0.0340	0.9035	0.9342	0.9189	0.9549	0.8775	0.8512
2	59	-8.67	-16	12.34	-5.10	10.05	6.68	0.0417	0.0384	0.9199	0.9260	0.9230	0.9557	0.8821	0.8556
3	90	3.62	-17	12.33	-4.95	6.13	4.08	0.0407	0.0395	0.9218	0.9240	0.9229	0.9559	0.8822	0.8557
4	120	14.59	-4	12.46	-6.90	16.11	10.66	0.0445	0.0358	0.9148	0.9309	0.9229	0.9552	0.8816	0.8552
5	151	21.90	3	12.53	-7.80	23.18	15.21	0.0498	0.0312	0.9051	0.9395	0.9223	0.9544	0.8803	0.8539
6	181	23.18	2.5	12.52	-7.80	24.39	15.98	0.0510	0.0302	0.9029	0.9414	0.9222	0.9543	0.8800	0.8536
7	212	18.17	-3	12.47	-7.05	19.45	12.83	0.0466	0.0338	0.9109	0.9346	0.9228	0.9549	0.8812	0.8548
8	243	8.10	-6	12.44	-6.60	10.43	6.93	0.0419	0.0383	0.9196	0.9262	0.9229	0.9557	0.8820	0.8555
9	273	-3.82	0	12.47	-7.35	8.28	5.51	0.0411	0.0389	0.9210	0.9251	0.9231	0.9558	0.8823	0.8558
10	304	-15.06	8	12.58	-8.70	17.34	11.46	0.0452	0.0351	0.9135	0.9322	0.9229	0.9551	0.8815	0.8551
11	334	-21.97	16	13.06	-15.90	26.89	17.55	0.0537	0.0281	0.8981	0.9453	0.9217	0.9539	0.8792	0.8528
12	365	-23.09	11	13.01	-15.15	27.39	17.86	0.0543	0.0277	0.8970	0.9461	0.9216	0.9538	0.8790	0.8526

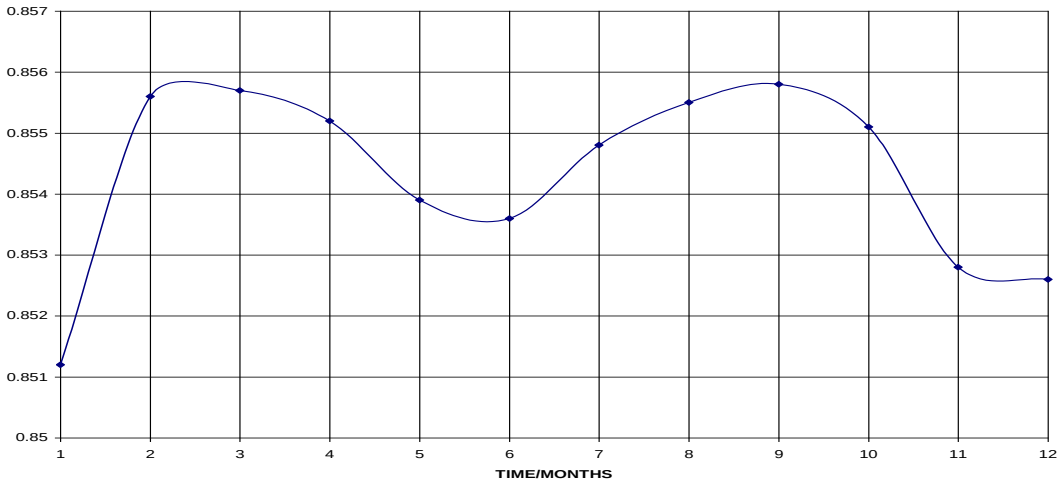

Fig. 2, variation of transmissivity with months of the year

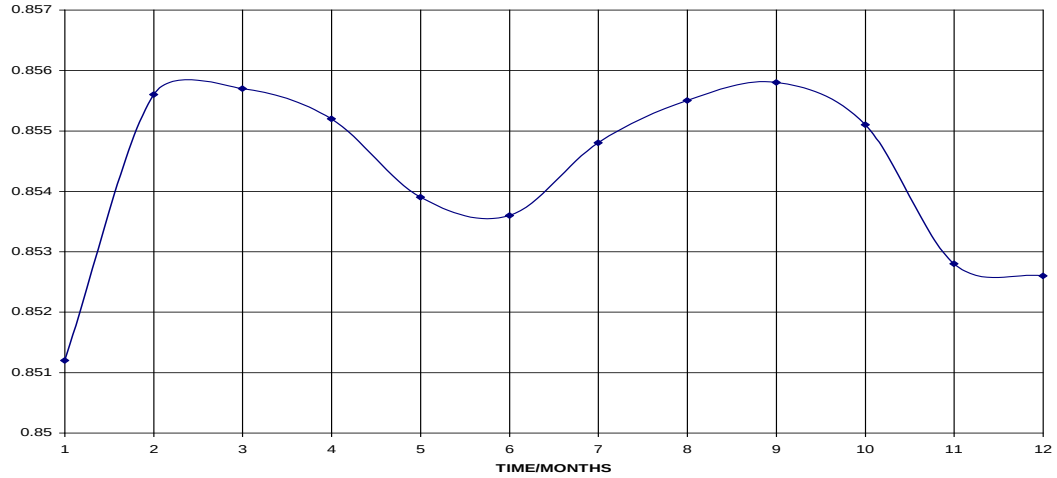

Fig. 3. variation of $(\tau\alpha)$ for a dull black surface

12/15/2010

Utility Mapping with Ground Penetrating Radar: an Innovative Approach

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Abstract: A new approach for the fitting of hyperbolic signatures due to point or cylindrical reflector in a GPR radargram is proposed. The technique is based on the least square error minimization of hyperbolic function derived from the general equation of hyperbola leading to the determination of the optimal values of the fitting parameters at the minimal level of sum of squared error function. The parameters are used to determine the radar velocity, the dielectric constant of the medium and the depth of the reflector. A test for the effectiveness of the proposed technique was conducted using a GPR radargram obtained at a road side where subsurface utilities are anticipated. A unique hyperbolic signature obtained in the radar image was digitized and interpreted using the developed algorism in MATLAB environment. Hyperbolic fitting parameters *a* and *b* were numerically obtained as 49.6444ns and 4.3182m respectively. The parameters were used to obtain the media velocity, dielectric constant and depth of the reflector as 0.174m/ns, 2.973 and 2.61m respectively. The technique therefore seems promising and a new approach to utility mapping. [Journal of American Science 2011;7(1):644-649]. (ISSN: 1545-1003).

Keywords: Ground penetrating radar, least square fitting, radar velocity, hyperbolic reflection, utility mapping

1. Introduction

Electromagnetic method of geophysical prospecting is widely applicable in mineral exploration and environmental studies. There are different techniques of the method but the most commonly used in engineering and environmental studies is the Ground Penetrating Radar (GPR). The term Ground Penetrating Radar or ground probing radar refers to a range of electromagnetic techniques designed primarily for the location of objects or interfaces buried beneath the earth surfaces or located within a visually opaque structure (Daniels, 2004). Ground-Penetrating Radar (GPR) has become a useful and efficient instrument for gathering information about subsurface geologic formations and detection of buried objects. GPR records continuous graphic profiles of the subsurface interfaces with high degree of accuracy. It is particularly found to be successful in detecting subsurface geologic formations, buried archeological remains, geologic subsurface fracture zones and cavities etc.

The range of application of GPR has been expanding steadily with the development of more sophisticated computing devices. The technique is successfully found to be applicable in stratigraphic studies of sedimentary formation (Bristow & Jol, 2003), outlining the foundation of building and other engineering structures, (Abbas, *et al*, 2009), archeological investigation (Negri, Leucci & Mazzone, 2008), location of water table, and characterization of subsurface contamination (Hamzah, Ismail & Samsudin 2009), geomorphic controls of flood-plain and surface subsidence (Poole *et al*, 2002), road inspection (Loizos & Plati, 2007), mine detection (Bruschini *et al*, 1998) etc.

One of the most usedful application of GPR in urban infrastructural engineering is mapping and detection of buried pipes. This unique application becomes imperative due to the ever growing urbanization in both developed and developing nations with its attendant demand for buried utilities. The construction, development and management of subsurface infrastructures has become a very viable business that attracts the attention of scientists and engineers over the past few years. Various utilities such as telecommunication and electric power cables,water and gas supply cables etc are delivered through underground pipes of varius sized buried at different depths. In many cases, maintainance of these infrastructures require digging operation which leads to unintentional damage to some of the facilities. Since most of these pipes are distinguishable from their depths and sizes, geophysical methods can be used to reduce the cost and effect of these damages. Various geophysical methods are known for their ability to determine the

overburden thickness and map subsurface conditions prior to excarvation and construction (Lukumon, Festus & Bolaji, 2010). GPR is relatively a new geophysical utility location tool for accurate mapping of various underground utilities. The technique provides a rapid, high resolution and non-inverssive means of identifying and characterizing underground pipes of different sizes at different depths.

Mapping underground utility requires the knowledge of the electromagnetic properties of the subsurface soil. The fundamental electromagnetic property of the subsurface soil is the radar propagation velocity across the soil medium. A radar pulse transmitted through a homogeneous medium propagates with a unique characteristic velocity that defines the medium. Radar velocity *v* is related to the relative dielectric permittivity of the medium ε_r by the equation

$$v = c / \sqrt{\varepsilon_r} \qquad 1$$

where *c* is the velocity of light in free space.

The propagation of radar signal in ground therefore depends on the dielectric permittivity of the soil. The dielectric permittivity of a material is a frequency-dependent response of the material to electromagnetic waves (Chu, *et al*, 2006) and it can be used to distinguish it from other materials. Velocity information with respect to a particular subsurface structure can therefore be used to detect variation or discontinuity within the media of different dielectric property.

Techniques of GPR radar velocity estimation are related to data collection modes. There are two types of data collection modes: the Common Offset (CO) mode in which distance between transmitting and receiving antennas is fixed and the entire system is pushed on a cart vehicle along a survey line. This mode has the advantage of being faster in data recording process and produce high resolution image of the subsurface. It is however difficult to estimate velocity from the data obtained using the mode (Nakashima, Zhou & Sato, 2001). The common mid-point (CMP) is the method commonly used in estimating the radar velocity. In this mode, commonly known as bistatic, the transmitter–receiver offset is increased in steps at either site along the profile beginning with the smallest offset. In the CMP gather, all the receiver traces are reflections from the same depth point which is directly beneath the centre of the spread. Tillard & Dubois (1995) reviewed the propagation equation for a two-way bistatic travel time of the reflected waves from a CMP gather, leading to a hyperbolic travel-time curve.

Most of the available GPR equipment are monostatic in which the two antennas are housed in a single casing with a fixed separation. A CMP survey cannot be conducted with such instrument. The most practicable technique for radar velocity estimation in this case is fitting the hyperbolic signature pattern due to a point or cylindrical reflector (Aitken & Steward, 2004). This involves the fitting of the hyperbolic spread due to the reflector with a mathematical model to determine the model parameters. The parameter model that minimizes error criterion can be used to simultaneously estimate the radar propagation velocity of the medium and the radius of the reflector (Ristic, Petrovacki & Govedarica, 2009).

It is however observed that the degree of accuracy with which the velocity can be determined from the model parameter is a subject of concern to many near surface geophysicists. This is mainly due to the fact that the position of the centre of the hyperbola for point reflector is different from that of a cylindrical reflector of finite radius R (Ristic *et al,* 2009). Point reflector is actually a special case of cylindrical reflector with radius R=0. The variation in the shape of the hyperbola would lead to the false assumption that the spread of the hyperbola is caused by higher magnitude of velocity and can consequently lead to an incorrect velocity value. Thus a polynomial fitting of the hyperbola do not adequately characterized the hyperbola in terms of the model parameters and therefore failed to provide the necessary information for target identification. In other word, second order least square polynomial fitting of the hyperbolic signatures cannot be used to accurately estimate the radar velocity especially if the hyperbolic reflection signature is due to a cylindrical object of finite non zero radius. In most engineering applications especially in urban areas, the reflection is due to buried utility pipes of none zero radius. The specific position, depth and the dielectric constant of the surrounding medium are vital information that cannot be compromised.

In an attempt to overcome this limitation, Shihab & Al-Nuaimy (2005) developed and presented a direct least square method that is specifically adopted for conic section in which the constraints on the parameter vectors were modified to match the properties of a hyperbolic conic section. The method, which is based on quadratic constrained least square fitting, is an extension of an efficient technique for fitting ellipse to scattered data points

developed by Fitzgibbon, Pitu & Fisher (1999). They consider the ellipse specific property constrain into the normalization factor by minimizing the algebraic distance based on the following specific properties of conic sections:

$4ac - b^2 = 1$ parabolic ,
$4ac - b^2 > 1$ hyperbolic ,
$4ac - b^2 < 1$ ellipse.

where *a, b,* and *c* are the fitting parameters of the conic sections. Detail description of the technique was presented by O'Leary & Zsombor-Murray (2004). The technique was recently utilized by Ristic *et al* (2009) to directly estimate radar propagation velocity and cylindrical object radius with an optimality criterion that minimizes the sum of squares of the residuals. They estimated the velocity iteratively by varying its magnitude from minimum to maximum possible values in constant steps. The value closest to satisfying the optimality criterion which minimized the sum of squared residuals is accepted as the estimated velocity and used to determine the depth of the reflector.

In this work, a similar but more direct approach for determination of the radar velocity and depth of reflector is proposed. The algorithm for the proposed technique was derived from the general equation of an ideal vertical transverse axis hyperbola in line with the appearance of the hyperbolic signature due to point or cylindrical reflector in a GPR radargram. The proposed technique also fits the hyperbola using the least-square minimization of error function that is directly executable leading to the optimal values of the hyperbolic parameters. The technique was a modification of the fitting procedure developed by Chaudhuri (2010) for fitting circles and ellipses in target detection using the boundary points of the image region. The hyperbola–constrained least square fitting algorithm was proposed and tested on field trial data. The theoretical frame work is discussed below.

2. Model geometry

Consider a simple geometry like a horizontal cylinder buried in a homogeneous medium on a plane perpendicular to the direction of motion of the antennas (Fig. 1). It could be observed from the figure that

$$(Z + R)^2 = (X_i - X_0)^2 + (Z_0 + R)^2 \qquad 2$$

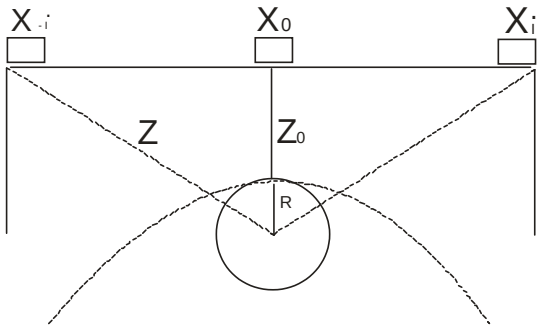

Fig. 1, hyperbolic signature spread due to buried cylinder

Obviously the depth to the top of the cylinder Z is given by

$$Z_0 = \frac{vt_0}{2}$$

and the apparent depth Z when the antennas are at X_i (or X_{-i}) is

$$Z = \frac{vt}{2}$$

Substituting the above in equation 2 and rearranging, we have

$$\left(\frac{t+\frac{2R}{v}}{t_0+\frac{2R}{v}}\right)^2 - \left(\frac{x-x_0}{\frac{v}{2}t_0+R}\right)^2 = 1 \qquad 3$$

Equation 3 defines a hyperbola of semi axes *a* and *b* given by

$$\frac{(x-x_0)^2}{a^2} - \frac{(y-y_0)^2}{b^2} = 1 \qquad 4$$

Where

$$a = t_0 + \frac{2R}{v} \qquad 5$$

$$b = \frac{v}{2}t_0 + R \qquad 6$$

Eliminating *v* in 5 and 6, we have

$$v = \frac{2b}{a} \qquad 7$$

Thus it is possible to estimate the velocity and hence the depth of the reflector from the hyperbola parameters *a* and *b*. These parameters can be obtained using the least square polynomial curve

fitting of the hyperbolic signatures due to the cylindrical reflector. It could be observed from equation 3 that for a point reflector (R=0),

$$\left(\frac{t}{t_0}\right)^2 - \left[2\left(\frac{x-x_0}{vt_0}\right)\right]^2 = 1 \qquad 8$$

This implies variation in the position of the centre of the hyperbola. The hyperbola, which was centered around *2R/v* for R>0 is now shifted to (x_o,*0*). This leads to the false assumption that the spread of the hyperbola is affected by a higher value of propagation velocity while in actual sense, velocity is only a function of material dielectric and independent of the radius of the reflector.

Consider an ideal vertical transverse axis hyperbola of coefficients *a* and *b* centered at the origin. The equation for this hyperbola is

$$\frac{y_i^2}{a^2} - \frac{x_i^2}{b^2} = 1 \qquad 9$$

where (x_i, y_i), i =1, 2, 3,…n are the n-point coordinates of the points along the curve. If the curve is a perfect hyperbola, then all the points (x_i, y_i) satisfy equation 9 and thus the error due to fitting of the hyperbola is zero. For real field hyperbolic signatures in a radargram, the coordinates of the curve may not perfectly lie on the fitting hyperbola. For any point (x_i, y_i) on the curve, the error generated *e* is given by the difference between the left and the right hand sides of equation 9. That is

$$\left(1 - \frac{y_i^2}{a^2} + \frac{x_i^2}{b^2}\right) \qquad 10$$

The error due to n points is therefore the sum of all the n point errors given by

$$e = \sum_{i=1}^{n}\left(1 - \frac{y_i^2}{a^2} + \frac{x_i^2}{b^2}\right)$$

The square error for all the n-points e^2 is

$$e^2 = \sum_{i=1}^{n}\left(1 - \frac{y_i^2}{a^2} + \frac{x_i^2}{b^2}\right)^2 \qquad 11$$

The above equation is a function of the parameters *a* and *b*. The parameters are to be determined such that the square error e^2 (same as the sum of squared residuals $SS_{residual}$) is minimized. The optimal values of *a* and *b* are obtainable by differentiating e^2 with respect to the parameters and equating the differentials to zero. That is by solving the equations

$\frac{\partial e^2}{\partial a} = 0;$ leading to

$$\sum_{i=1}^{n} y_i^2 - \sum_{i=1}^{n}\frac{y_i^4}{a^2} + \sum_{i=1}^{n}\frac{x_i^2 y_i^2}{b^2} = 0 \qquad 12$$

And

$\frac{\partial e^2}{\partial b} = 0;$

leading to

$$\sum_{i=1}^{n} x_i^2 - \sum_{i=1}^{n}\frac{x_i^2 y_i^2}{a^2} + \sum_{i=1}^{n}\frac{x_i^2}{b^2} = 0 \qquad 13$$

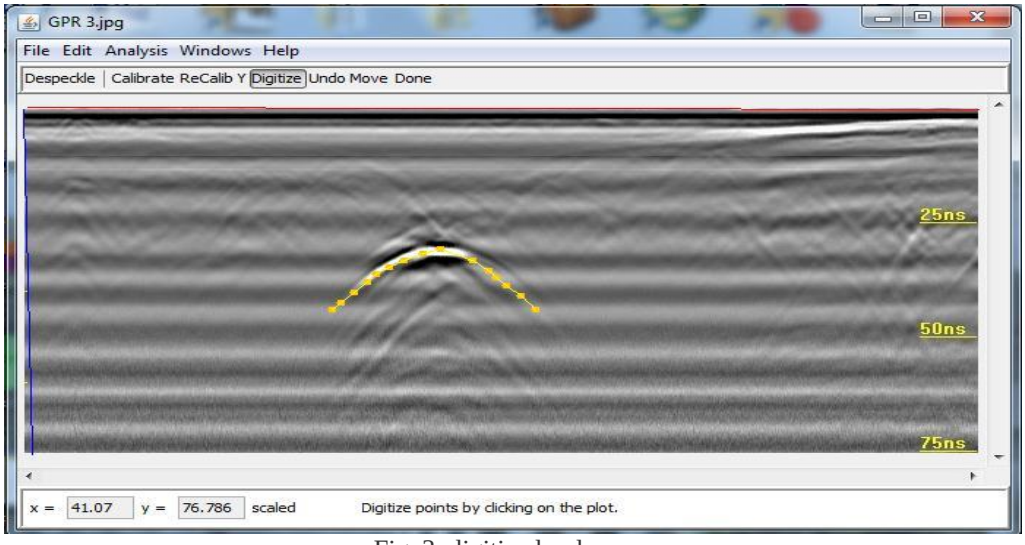

Fig. 3, digitized radar scan

Equations 12 and 13 can be solve for *a* and *b* leading to the following equations

$$a^2 = \frac{\sum_{i=1}^{n} x_i^4 \sum_{i=1}^{n} y_i^4 - \left(\sum_{i=1}^{n} x_i^2 y_i^2\right)^2}{\sum_{i=1}^{n} x_i^4 \sum_{i=1}^{n} y_i^2 - \left(\sum_{i=1}^{n} x_i^2 y_i^2\right)\sum_{i=1}^{n} \quad (14)$$

And

$$b^2 = \frac{\left(\sum_{i=1}^{n} x_i^2 y_i^2\right)\sum_{i=1}^{n} x_i^4 - \left(\sum_{i=1}^{n} x_i^2 y_i^2\right)^2}{\sum_{i=1}^{n} y_i^2 \sum_{i=1}^{n} x_i^2 y_i^2 - \sum_{i=1}^{n} x_i^2 \sum_{i=1}^{n} y_i^4} \quad (15)$$

Thus the optimum values of the parameters can easily be computed with the coordinates (x_i, y_i) as inputs.

3. Method and materials

A GPR cross section was obtained on a trial field over a buried pipe along Jalan Tampoi road site in Johor Bahru using a multichannel IDS DAD fast wave radar acquisition unit. The acquired data is preprocessed with GRED IDS 3D software. The processed radargram was used as a sample data for the assessment of the performance of the above algorithm.

The coordinates of the hyperbolic signatures were recorded with a plot digitizer 2.4.1, (Fig. 3), a Java program used to digitize scanned plots of functional data developed by Huwaldt (2005).The algorithm was executed in a MATLAB environment with the following implementation code.

```
function [a,b]=hypfit(x,t)
%Filename: hypfit.m
%Usage: [a,b]=hypfit(x,t)
%Input
% x horizontal distance coordinates (m)
% t vertical time coordinates (ns)
%Output
% a fitting coefficient (a)
% b fitting coefficient (b)
P=sum(x.^2);
Q=sum(t.^2);
R=sum(x.^4);
S=sum(t.^4);
T=sum((x.^2).*(t.^2));
a=sqrt((R.*S-T.^2)/(R.*Q-T.*P));
b=sqrt((T.*R-T.^2)/(Q.*T-P.*S));
end
```

4. Result and discussion

The results yield a numerical values of the fitting coefficients for the hyperbolic signature *a* and *b* as 49.6444ns and 4.3182m respectively. The parameters are used to estimate the velocity (equation 7) and a numerical value of 0.174m/ns is obtained. The dielectric permittivity of the soil is computed using equation 1 and a value of 2.973 is obtained. The velocity and the two-way travel time obtained from the radargram (Fig. 3) are used to compute the depth of the reflector and a value of 2.61m is obtained.

The study area is a site of a tarred road in a commercial area within the northern part of Johor Bahru, Malaysia. The site experienced series of sand filling and compaction over the years as a result of infrastructural development. Subsurface utilities are therefore likely to be found at various depths due to long time of human activities. Thus at a depth of 2.61m, the reflector is suspected to be an age long forgotten pipe buried before the development of the road to its presence state. Even though lateritic soil is clearly visible within the edge of the study area, the relatively small magnitude of the dielectric constant (2.973) suggests that the pipe is likely buried within a deeper soil horizon of relatively low dielectric constant, most likely dry clay or sand overplayed by a thin layer of lateritic soil cover. The lateritic soil cover appears as first strong reflection in the GPR cross section (Fig. 3).

5. Conclusion

A new approach for the fitting of hyperbolic signatures due to point or cylindrical reflector in GPR radargram is presented. The technique is a modification of the fitting procedure developed by Chaudhuri (2010) for fitting circles and ellipses based on the least square error minimization of hyperbolic function. With hyperbola-constrained fitting, the optimal values of the fitting parameters are determined at the minimal level of sum of squared error. The parameters are used to determine the radar velocity and dielectric constant of a soil medium as well as the depth of buried cylindrical pipe. The technique is used to detect a deeply buried utility at a depth of 2.61m within a subsurface soil of dielectric permittivity 2.973. Modeling and further testing of the technique will no doubt enhance the effectiveness

of the application of GPR in underground utility mapping.

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Application of variational iteration method for solving the nonlinear generalized Ito system

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Abstract: In this article, we implement relatively analytical technique called the variational iteration method (VIM)for solving nonlinear generalized Ito system. In this method, a correction functional is constructed by a general Lagrange multiplier. Two cases are given to illustrate the accuracy and effectiveness of the method .We compare our results with results obtained by exact solution. This Comparison reveals that the variational iteration method is very effective, convenient and easier to be implemented.
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1. Introduction

In this paper, we extend the application of the variatioal iteration method to find approximate solutions for nonlinear generalized Ito system. The variational iteration method, which proposed by Ji-Huan He [1-3], is considered to find analytic and approximate solutions of differential equations. It's effectively and easily used to solve some classes of nonlinear problems, Variational iteration method has been favorably applied to various kinds of nonlinear problems. The main property of the method is in its flexibility and ability to solve nonlinear equations accurately and conveniently. Major applications to nonlinear wave equation, nonlinear fractional differential equations, nonlinear oscillations and nonlinear problems arising in various engineering applications are surveyed. The flexibility and adaptation provided by the method have made the method a strong candidate for approximate analytical solutions.

2. Variational Iteration method

To illustrate the basic concepts of the Variatioal iteration method, we consider the differential equation in the formal form

Lu +**N**u=**g**(x)**,**

where L is a linear operator, N a nonlinear operator and g(x) an inhomogeneous term. According to VIM, we can construct a correctional functional as

$$u_{n+1}(x) = u_n(x) + \int_0^x \lambda\{Lu_n(\xi) + N\overline{u_n(\xi)}\}d\xi ,$$

where λ is a Lagrangeian multiplier [4], which can be determined by using variational theory, the subscript n denotes the n-th order approximation, and $\overline{u_n(\xi)}$ is considered as a restricted variational, i.e. $\overline{u_n(\xi)}$ =0.

3. Application

Consider the nonlinear generalized Ito system of partial differential equations [5]

$$u_t = v_x , \quad (1)$$
$$v_t = -2(v_{xxx} + 3\,u\,v_x + 3\,v\,u_x) - 12\,w\,w_x + 6p_x , \quad (2)$$
$$w_t = w_{xxx} + u\,w_x , \quad (3)$$
$$p_t = p_{xxx} + u\,p_x . \quad (4)$$

To illustrate the degree of accuracy to VIM, two cases of nonlinear generalized Ito system exact are discussed in details.

3.1. Nonlinear generalized Ito system case 1

In this case the analytical solution for system (1-4)

$$u(x,t) = a_0 - 2k^2m^2\,Sn^2(\xi) , \quad (5a)$$

$$v(x,t) = -\frac{1}{2k^2m^2}(3k^6m^2(1+m^2)^2 - 12k^4m^2(1+m^2)a_0 + 9k^2m^2a_0^2 - c_1^2) + 2k^4m^2(1+m^2) - 6k^2m^2a_0)Sn^2(\xi) \quad (5b)$$

$$w(x,t) = c_0 + c_1\,Sn(\xi) , \quad (5c)$$
$$p(x,t) = e_0 + c_0c_1\,Sn(\xi) . \quad (5d)$$

where a_0, k, m, c_0, c_1 and $e_0$are constant, and $\xi = k\,x + (-k^3(1+m^2)+3ka)\,t + \xi_0$, and ξ_0 is constant. we start with initial approximation $u_0 = u(x,0)$, $v_0 = v(x,0)$, $w_0 = w(x,0)$ and $p_0 = p(x,0)$ given by

$$u_0(x,t) = a_0 - 2k^2m^2\,Sn^2(k\,x + \xi_0) , \quad (6a)$$

$v_0(x,t) =$

$$-\frac{1}{2k^2m^2}(3k^6m^2(1+m^2)^2 - 12k^4m^2(1+m^2)a_0 + 9k^2m^2a_0^2 - c_1^2)Sn^2(kx+\xi_0)$$

$$w_0(x,t) = c_0 + c_1\, Sn(kx+\xi_0), \quad (6c)$$

$$p_0(x,t) = e_0 + c_0c_1\, Sn(kx+\xi_0). \quad (6d)$$

To solve the system (1-4) by means of variational iteration method, we can construct the correct functional as follows:

$$u_{n+1}(x,t) = u_n(x,t) + \int_0^t \lambda_1\{u_{nt} - \overline{v_{nx}}\}d\tau, \quad (7a)$$

$$v_{n+1}(x,t) = v_n(x,t) + \left[\int_0^t \lambda_2\{v_{nt} + 2(v_{nxxx} + 3\overline{u_n v_{nx}} + 3\overline{v_n u_{nx}}) + 12\overline{w_n w_{nx}} + 6\overline{p_{nx}})\}d\tau\right] \quad (7b)$$

$$w_{n+1}(x,t) = w_n(x,t) + \int_0^t \lambda_3\{w_{nt} - w_{nxxx} - 3\overline{u_n w_{nx}}\}d\tau, \quad (7c)$$

$$p_{n+1}(x,t) = p_n(x,t) + \int_0^t \lambda_3\{p_{nt} - p_{nxxx} - 3\overline{u_n p_{nx}}\}d\tau. \quad (7d)$$

where λ_1, λ_2, λ_3 and λ_4 are Lagrange multipliers are to determined, and $\overline{v_{nx}}$, $\overline{u_n v_{nx}}$, $\overline{v_n u_{nx}}$, $\overline{w_n w_{nx}}$, $\overline{p_{nx}}$, $\overline{u_n w_{nx}}$ and $\overline{u_n p_{nx}}$ are denotes restricted variations, **i.e.** $\delta\overline{v_{nx}} = 0$, $\delta\overline{u_n v_{nx}} = 0$, $\delta\overline{v_n u_{nx}} = 0$, $\delta\overline{w_n w_{nx}} = 0$, $\delta\overline{p_{nx}} = 0$, and $\delta\overline{u_n p_{nx}} = 0$. Its stationary conditions can be obtained as follows :

$$1 + \lambda_1|_{\tau=t} = 0, \quad \lambda_1' = 0 \Rightarrow \lambda_1 = -1, \quad (8a)$$

$$1 + \lambda_2|_{\tau=t} = 0, \quad \lambda_2' = 0 \Rightarrow \lambda_2 = -1, \quad (8b)$$

$$1 + \lambda_3|_{\tau=t} = 0, \quad \lambda_3' = 0 \Rightarrow \lambda_3 = -1, \quad (8c)$$

$$1 + \lambda_4|_{\tau=t} = 0, \quad \lambda_4' = 0 \Rightarrow \lambda_4 = -1. \quad (8d)$$

substituting (8a - 8d) in (7a - 7d), and the following variational iteration formula can be obtained

$$u_{n+1}(x,t) = u_n(x,t) - \int_0^t \{u_{nt} - \overline{v_{nx}}\}d\tau, \quad (9a)$$

$$v_{n+1}(x,t) = v_n(x,t) - \int_0^t \{v_{nt} + 2(v_{nxxx} + 3\overline{u_n v_{nx}} + 3\overline{v_n u_{nx}}) + 12\overline{w_n w_{nx}} + 6\overline{p_{nx}})\}d\tau, \quad (9b)$$

$$w_{n+1}(x,t) = w_n(x,t) - \int_0^t \{w_{nt} - w_{nxxx} - 3\overline{u_n w_{nx}}\}d\tau, \quad (9c)$$

$$p_{n+1}(x,t) = p_n(x,t) - \int_0^t \{p_{nt} - p_{nxxx} - 3\overline{u_n p_{nx}}\}d\tau. \quad (9d)$$

with $\boldsymbol{n \geq 0}$.

By the above iteration formulas (9a - 9d),we can obtain directly the order components as

$u_1(x,t) = a_0 + 4\ k(3a_0\ k^2m^2 + k^4m^2 + k^4m^4)\ t\ Cn(kx+\xi_0)\ Dn(kx+\xi_0)\ Sn(kx+\xi_0) - 2\ k^2m^2\ Sn^2(kx+\xi_0)$,

$v_1(x,t) = 1/2k^2m^2\ \{\ c_1^2 - 9\ a_0^2\ k^2\ m^2 + 12\ a_0\ k^4\ m^2 - 3\ k^6\ m^2 + 12\ a_0\ k^4\ m^4 - 6k^6\ m^4 - 3\ k^6\ m^6 + 64\ k^7\ m^5(-3\ a_0 + k^2\ (1+m^2))tCn^3\ (kx+\xi_0)\ Dn\ (kx+\xi_0)\ Sn\ (kx+\xi_0) + 4k^4\ m^4(-3\ a_0 + k^2(1+m^2))Sn^2\ (kx+\xi_0) - 8\ k^5\ m^4\ t\ Cn\ (kx+\xi_0)\ Dn\ (kx+\xi_0)\ Sn\ (kx+\xi_0)(9\ a_0^2 - 30\ a_0\ k^2 + 9\ k^4 - 30\ a_0\ k^2\ m^2 + 18\ k^4\ m^2 + 9\ k^4\ m^4 + 8(3\ a_0\ k^2 - k^4(1+m^2)\ Dn^2\ (kx+\xi_0) - 8\ k^2\ m(-1+3\ m)(-3\ a_0 + k^2(1+m^2))Sn^2\ (kx+\xi_0)\}$

$w_1(x,t) = c_0 - c_1\ k^3\ m\ t\ Cn^3(kx+\xi_0)\ Dn(kx+\xi_0) + c_1\ Sn(kx+\xi_0) + c_1\ k\ t\ Cn\ (kx+\xi_0)\ Dn\ (kx+\xi_0)\ (3\ a_0 - k^2\ Dn^2(kx+\xi_0) + 2\ k^2(2-3\ m)\ m\ Sn^2(kx+\xi_0)$,

$p_1(x,t) = e_0 + 2\ c_0\ c_1\ Sn(kx+\xi_0) - 2c_0\ c_1\ k^3\ t\ Cn(kx+\xi_0)\ Dn(kx+\xi_0)\ (m\ Cn^2(kx+\xi_0) + Dn^2(kx+\xi_0) - 4m\ Sn^2(kx+\xi_0)) + 6\ c_0c_1\ k\ t\ Cn(kx+\xi_0)\ Dn(kx+\xi_0)\ (a_0 - 2k^2m^2Sn^2(kx+\xi_0)$.

and so on, in the same manner using *Mathematica* Package, we can evaluate the numerical solutions to the rest components of iteration formulas (9a - 9d) with n th approximations (n =3). The obtained numerical results are summarized in Table 1.

Table 1. Comparison of the exact and numerical solutions for Ito system

	t	Error u	Error v	Error w	Error p
x= -200	0	0	-3.683E-12	0	0
	0.5	-2.6514E-11	3.6744E-10	4.5074E-08	9.0147E-08
	1	-1.8466E-10	2.9358E-09	1.2389E-07	2.4777E-07
	1.5	-5.2322E-10	9.9244E-09	2.3644E-07	4.7288E-07
	2	-9.9882E-10	2.352E-08	3.8273E-07	7.6547E-07
x= -100	0	0	3.683E-12	0	0
	0.5	4.3126E-11	-1.8335E-09	-5.0306E-08	7.181E-08
	1	3.6293E-10	-1.4574E-08	-6.8267E-08	1.2719E-07
	1.5	1.2867E-09	-4.9215E-08	-5.3864E-08	1.6631E-07
	2	3.1974E-09	-1.1666E-07	-7.078E-09	1.8931E-07
x= 0	0	0	-3.638E-12	0	0
	0.5	3.0864E-11	7.4125E-10	-8.8631E-08	-1.7726E-07
	1	1.8954E-10	5.9626E-09	-2.1134E-07	-4.2269E-07
	1.5	4.3654E-10	3.3138E-08	-3.6814E-07	-7.3627E-07
	2	5.4754E-10	7.854E-08	-5.5901E-07	-1.118E-06
x= 100	0	0	3.683E-12	0	0
	0.5	-6.7617E-11	1.2224E-09	4.5074E-08	9.0147E-08
	1	-5.1269E-10	9.8225E-09	1.2389E-07	2.4777E-07
	1.5	-1.6298E-09	-1.6298E-09	2.3644E-07	4.7288E-07
	2	-3.6218E-09	-3.6218E-09	3.8273E-07	7.6547E-07
x= 200	0	0	-3.683E-12	0	0
	0.5	7.4224E-11	-1.0587E-09	4.9735E-08	9.9471E-08
	1	6.2245E-10	-8.502E-09	6.5361E-08	1.3072E-07
	1.5	2.2033E-09	-2.8707E-08	4.6877E-08	9.3753E-08
	2	5.4691E-09	-6.8048E-08	-5.7178E-09	-1.1436E-08

From these results we conclude that the variational iteration method for Ito system, gives high degree of accuracy in comparison with analytical solution (5a-5d). Now we study the diagrams obtained by VIM and analytical solutions to show the relation between u,v,w,p and x with different values for t, and the relation between u,v,w,p and t with different values for x.

3.2. Nonlinear generalized Ito system *case* 2

In this case the analytical solution for system (1-4)

$$u(x,t) = a_0 - 2k^2 m^2\, Cs^2(\xi), \quad (10a)$$

$$v(x,t) = -\frac{1}{2k^2}(3k^6(2-m^2)^2 - 12k^4(2-m^2)a_0 + 9k^2a_0^2 - c_1^2) \quad (10b)$$
$$+2k^4(2-m^2) - 6k^2a_0)Cs^2(\xi)$$

$$w(x,t) = c_0 + c_1\, Cs(\xi), \quad (10c)$$

$$p(x,t) = e_0 + c_0c_1\, Cs(\xi). \quad (10d)$$

where a_0, k, m, c_0, c_1 and e_0 are constant, and $\xi = k\,x + (-k^3(1+m^2)+3ka)\,t + \xi_0$, and ξ_0 is constant. we start with initial approximation $u_0 = u(x,0)$, $v_0 = v(x,0)$, $w_0 = w(x,0)$ and $p_0 = p(x,0)$ given by

$$u_0(x,t) = a_0 - 2k^2 m^2\, Sn^2(k\,x + \xi_0), \quad (11a)$$

$$v_0(x,t) = -\frac{1}{2k^2}(3k^6(2-m^2)^2 - 12k^4(2-m^2)a_0 + 9k^2a_0^2 - c_1^2) \quad (11b)$$
$$+2k^4(2-m^2) - 6k^2a_0)Cs^2(k\,x+\xi_0)$$

$$w_0(x,t) = c_0 + c_1\, Sn(kx + \xi_0), \quad (11c)$$

$$p_0(x,t) = e_0 + c_0c_1\, Sn(kx + \xi_0). \quad (11d)$$

By using iteration formulas (9a - 9d),we can obtain directly case 2 order components as

$$u_1(x,t) = a_0 - 2k^2Cs^2(k\,x + \xi_0) + 4k^3(3a_0 - k^2(-2+m^2)\,t$$
$$Cs(k\,x + \xi_0)\,Ds(k\,x + \xi_0)\,Ns(k\,x + \xi_0).$$

$$v_1(x,t) = 1/(2k^2)\{c_1^2 - 3(3a_0^2k^2 - 4a_0k^4(-2 + m^2) + k^6(-2+m^2)^2) - 4(3a_0k^4 - k^6(-2 + m^2)\,Cs^2(k\,x + \xi_0) +$$

$128\ k^7(3\ a_0 - k^2(-2+m^2)\ t\ Cs^3(k\ x + \xi_0)Ds(k\ x + \xi_0)Ns(k\ x + \xi_0) + 8\ k^5\ t\ Cs(k\ x + \xi_0)Ds(k\ x + \xi_0)$

$Ns(k\ x + \xi_0)\ (9\ a_0^2 + 60\ a_0\ k^2 + 36\ k^4 - 30\ a_0 k^2\ m^2 - 36\ k^4\ m^2 + 9\ k^4 m^4 -$

$8(3\ a_0 k^2 - k^4(-2 + m^2)Ds^2(k\ x + \xi_0) - 8(3\ a_0\ k^2 - k^4(-2 + m^2)Ns^2(k\ x + \xi_0)\}$

$w_1(x,t) = c_0 + c_1\ Cs(k\ x + \xi_0) + 2\ c_1\ k^3\ t\ Cs^2(k\ x + \xi_0)Ds(k\ x + \xi_0)\ Ns(k\ x + \xi_0) - c_1\ k^3\ t\ Ds^3(k\ x + \xi_0)$

$Ns(k\ x + \xi_0) - c_1\ k\ t\ Ds(k\ x + \xi_0)\ Ns(k\ x + \xi_0)\ (3\ a_0 + k2\ Ns^2(k\ x + \xi_0))$

$p_1(x,t) = e_0 + 2\ c_0\ c_1 Cs(k\ x + \xi_0) + 4\ c_0\ c_1\ k^3 t\ Cs^2(k\ x + \xi_0)Ds(k\ x + \xi_0)\ Ns(k\ x + \xi_0) -$

$2\ c_0\ c_1\ k^3\ t\ Ds^3(k\ x + \xi_0)\ Ns(k\ x + \xi_0) - 2\ c_0\ c_1\ k\ t\ Ds(k\ x + \xi_0)\ Ns(k\ x + \xi_0)(3\ a_0 + k^2\ Ns^2(k\ x + \xi_0))$

and so on, in the same manner we can evaluate the rest components of iteration formulas (9a - 9d) with n th approximations (n =3).The obtained numerical results are summarized in Table 2. The behavior of the solution obtained by VIM and analytic solution are shown in Figs. (3a - 3d) and (4a - 4d). Now we study the diagrams obtained by VIM and analytical solutions to show the relation between u,v,w,p and x with different values for t, and the relation between u,v,w,p and t with different values for x. The behavior of the 3rd iteration obtained by VIM Fig[4a - 4d] and the analytic solution Fig[3a - 3d]. Then, the surfaces respectively show the solution u(x,t),v(x,t), w(x,t), p(x,t).

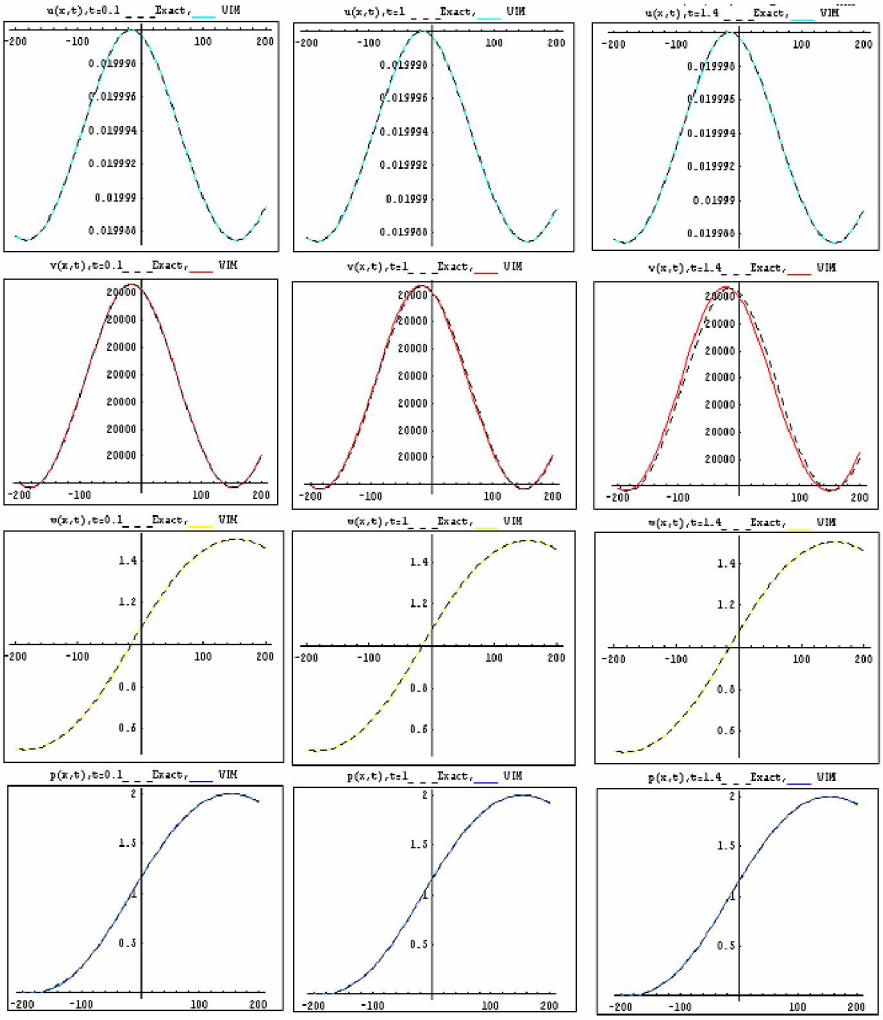


Fig show relation between u,v,w,p and x with constant values for t.

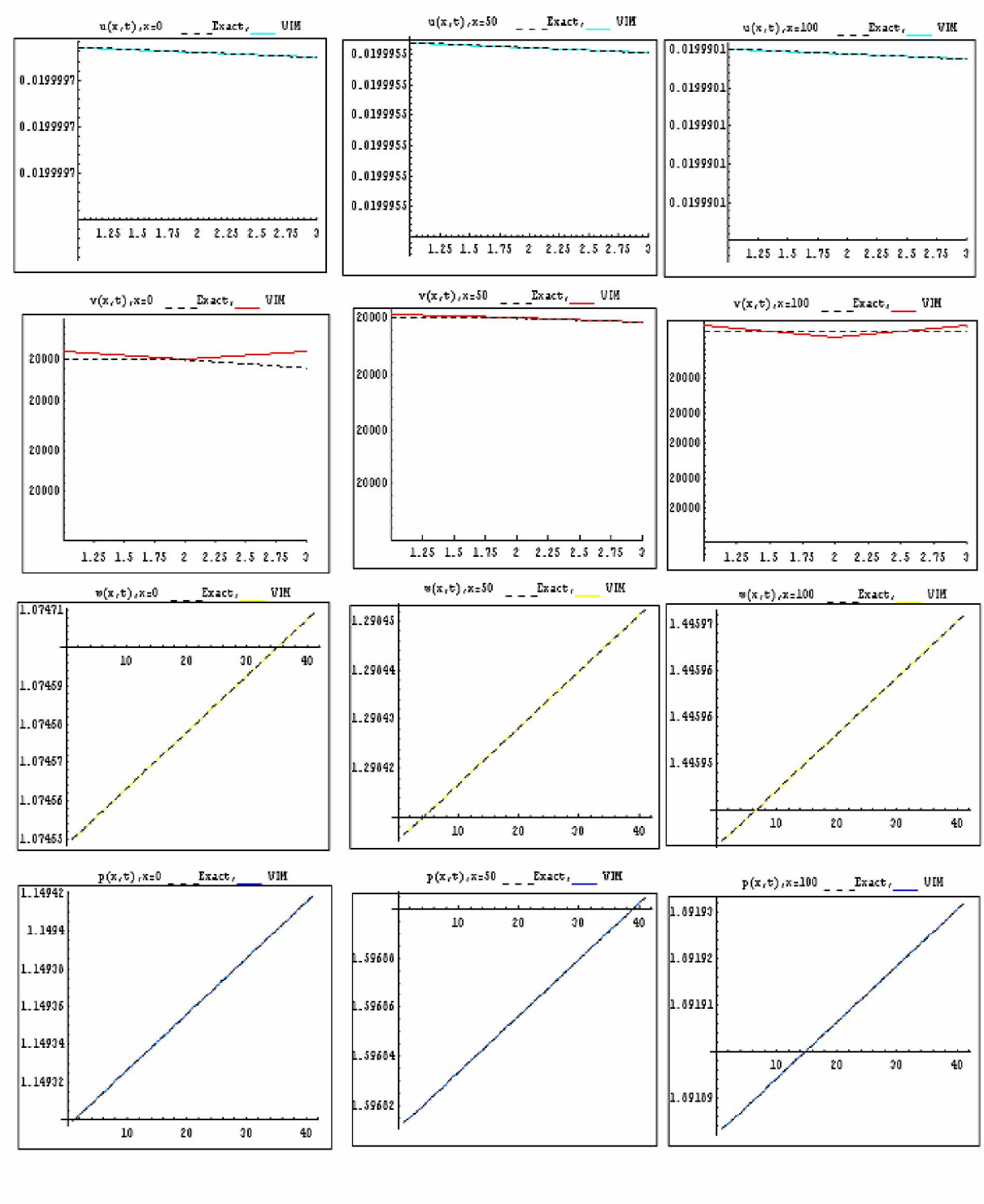
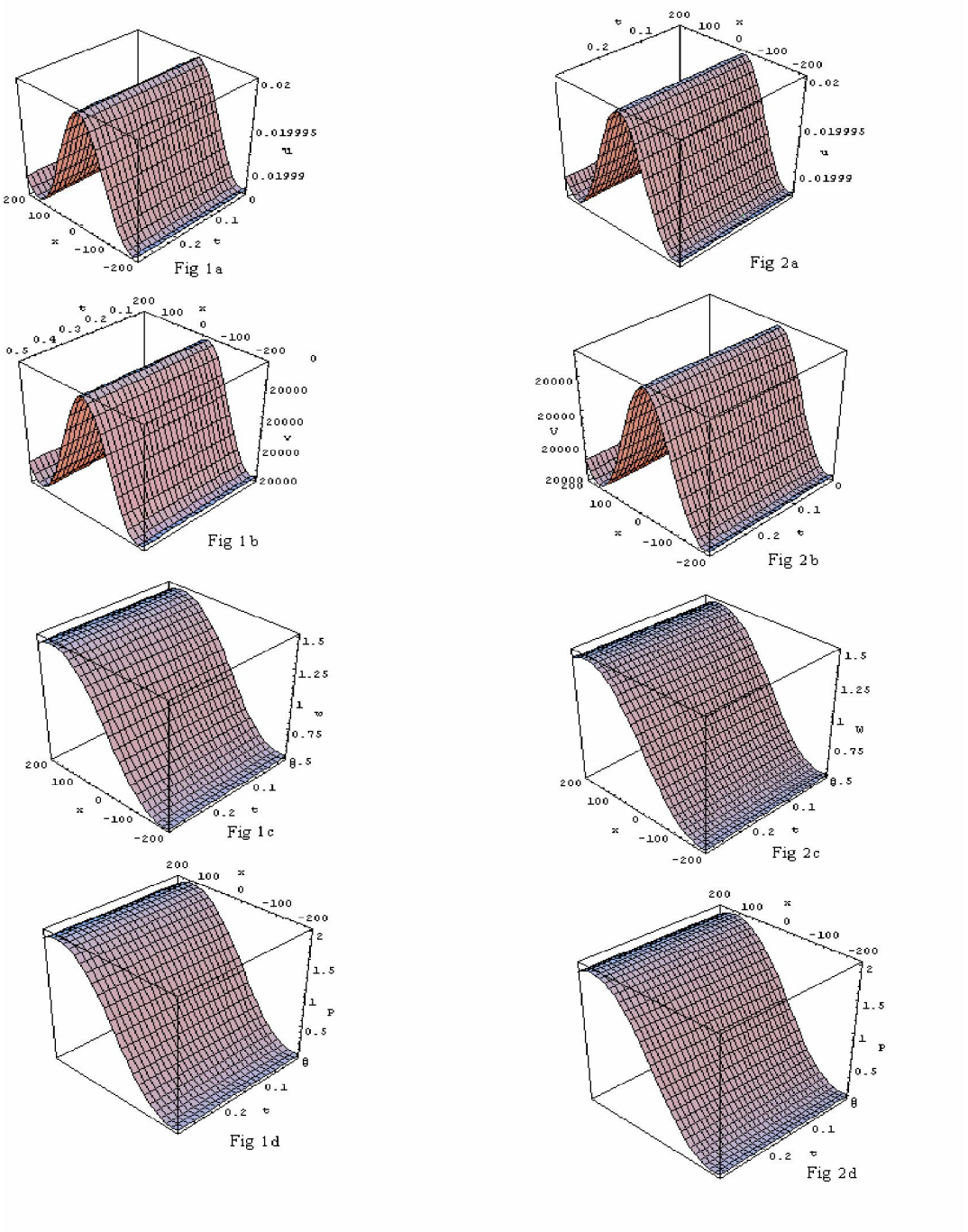


Fig show relation between u,v,w,p and t with constant values for x.



The behavior of the 3rd iteration obtained by VIM Fig[1a - 1d] and the analytic solution Fig[2a - 2d]. Then, the surfaces respectively show the solution u(x,t),v(x,t), w(x,t), p(x,t)

4- Conclusion

In this paper, the variational iteration method has been successfully used to find approximate solution for the nonlinear generalized Ito system of partial differential equation. The numerical results obtain using n approximations (n=3), compared with analytic solution show the high degree of accuracy.

Table 2. comparison of the 2nd case exact and numerical solutions for Ito system

	t	*Error u*	*Error v*	*Error w*	*Error p*
x= -200	*0*	0	0	0	0
	0.5	2.0061E-12	9.2723E-10	-3.5734E-08	-3.5734E-08
	1	1.593E-10	7.4278E-09	-5.9724E-08	-5.9724E-08
	1.5	8.869E-10	2.5063E-08	-7.1857E-08	-7.1857E-08
x= -120	*0*	0	0	0	0
	0.5	-5.0302E-11	-8.1702E-09	-9.4958E-08	-9.4958E-08
	1	2.7547E-10	-6.5469E-08	-2.614E-07	-2.614E-07
	1.5	2.7965E-09	-2.2111E-08	-4.9897E-07	-4.9897E-07
x= -40	*0*	0	0	0	0
	0.5	1.8093E-07	-8.5855E-06	-3.6379E-06	-3.6379E-06
	1	3.7522E-06	-0.000069122	-1.2928E-05	-1.2928E-05
	1.5	0.000020317	-0.00023475	-2.7729E-05	-2.7729E-05
x= 40	*0*	0	0	0	0
	0.5	3.6619E-09	1.7648E-07	1.0242E-07	1.0242E-07
	1	5.234E-08	1.4108E-06	7.5155E-07	7.5155E-07
	1.5	2.4907E-07	4.7559E-06	1.9522E-06	1.9522E-06
x= 120	*0*	0	0	0	0
	0.5	1.3132E-11	2.2646E-09	-3.17E-08	-3.17E-08
	1	3.1655E-10	-1.8146E-08	-3.6951E-08	-3.6951E-08
	1.5	1.6042E-09	6.1237E-08	-1.5605E-08	-1.5605E-08
x= 200	*0*	0	0	0	0
	0.5	-2.9265E-11	-3.9079E-09	-6.9876E-08	-6.9876E-08
	1	9.0988E-11	-3.1316E-08	-1.8075E-07	-1.8075E-07
	1.5	1.1602E-09	-1.0576E-07	-3.324E-07	-3.324E-07

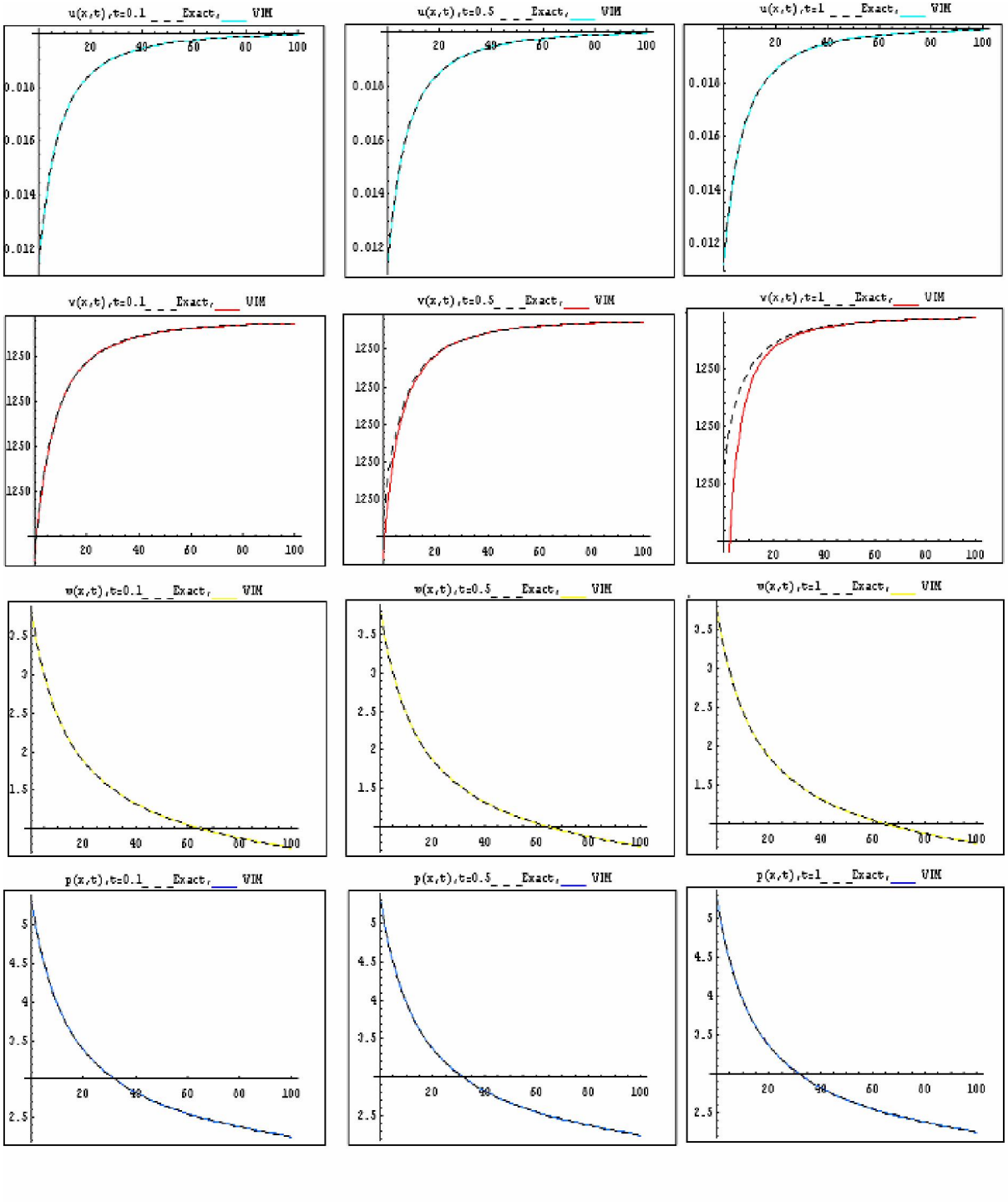


Fig show relation between u,v,w,p and x with constant values for t.

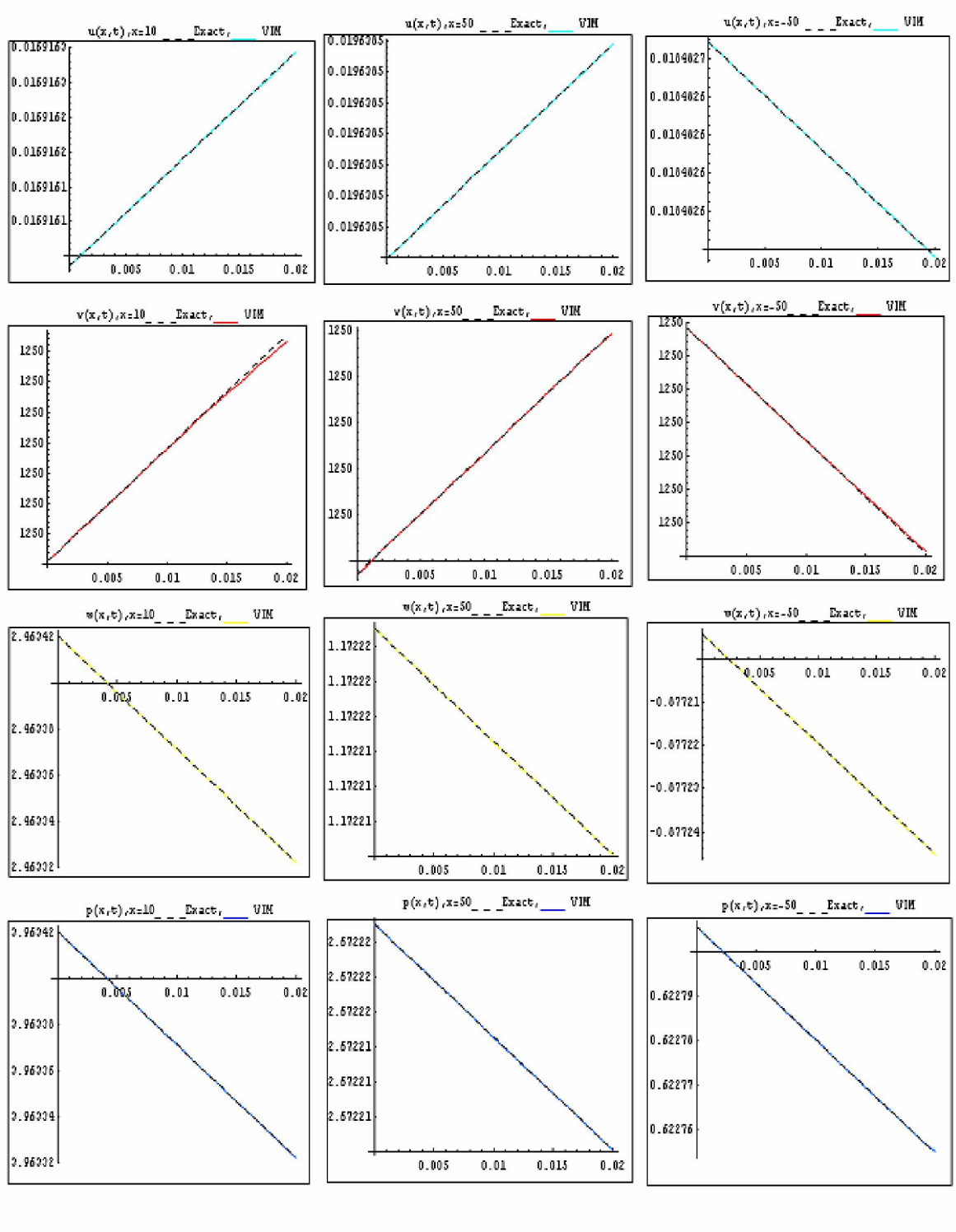
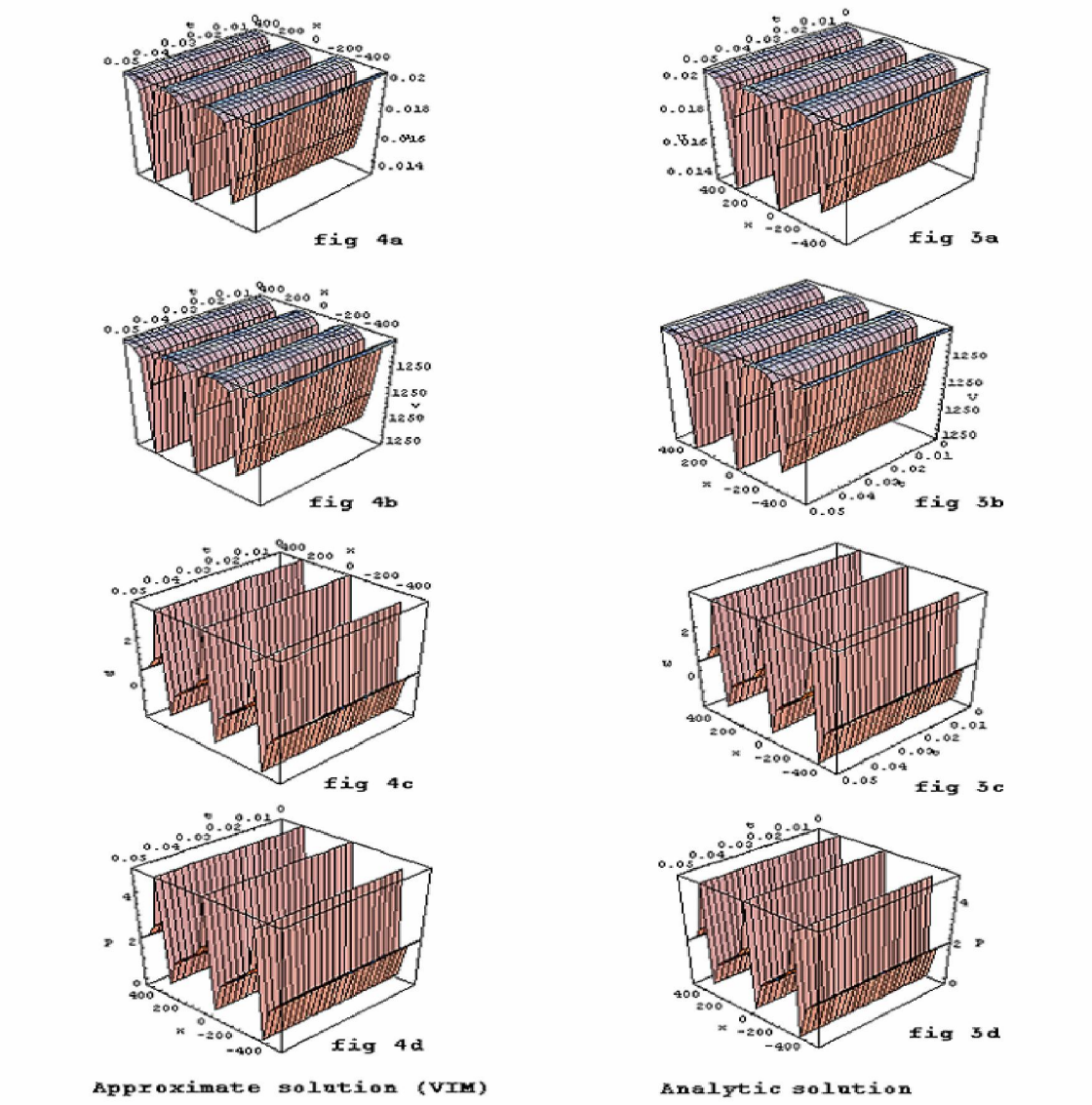


Fig show relation between u,v,w,p and t with constant values for x.



The behavior of the 3rd iteration obtained by VIM Fig[4a - 4d] and the analytic solution Fig[3a - 3d]. Then, the surfaces respectively show the solution u(x,t),v(x,t), w(x,t), p(x,t)

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Abundance Of Molluscs (Gastropods) At Mangrove Forests Of Iran

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ABSTRACT: This study determined the abundance and diversity of molluscs (focused on gastropod) at Hara Protected Area (HPA) and Gaz and Hara Rivers Delta (GHRD) mangroves, southern of Iran. Point count sampling method was employed in this study. A total of 1581 individual of gastropods, representing 28 species and 21 families, were observed in the two sites. The PCA plot indicated that all species have correlation with winter excluding species namely *Ethalia sp.*, *Haminoea sp.*, *Trichotropis sp.* and *Tibia insulaechorab curta* at HPA and *Telescopium telescopium*, *Stocsicia annulata*, and *Stenothyra arabica* at GHRD. The mean number of species was estimated 6.88±2.77 (per plot) versus 9.65±6.63 (per plot) at HPA and GHRD respectively. The results of X^2 test indicated that there was a high significant difference between total gastropod population observed at 4 seasons ($X^2_{3,1}$=31.9, p<0.001), but there was no significant difference in term of number of species between sites in order to seasonal observation ($X^2_{3,1}$=0.84, p>0.05). The results of diversity comparisons indicated that the highest diversity was in the HPA as compared to GHRD. Furthermore, the SIMPER analysis indicated that mangroves of HPA and GHRD were dominated with Asseminea sp., although the number of population was much higher at *R. mucronata* habitat. Eight species namely *Asseminea sp.*, *Stenothyra arabica*, *Cerithidium cerithinum*, *Littoria intermedia*, *Telescopium telescopium*, *Iravadia quadrasi*, *Atys cylindrica* and *Cyclostrema ocrinium* represented more than 91% of observations at HPA, while at GHRD, there were only three species namely *Asseminea sp.*, *Stenothyra arabica* and *Cerithidea cingulata* which represented more than 90% of observations. The result states that the great importance of HPA and GHRD for gastropod assemblages as main food resource for wading birds must be recognized and the protection of these sites from threats must be thoroughly enhanced.

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Key words: Gastropod, Mangrove Forest, Abundance, Iran

INTRODUCTION

The term molluscs refer to an ecological group of invertebrates that belong to many lesser known creatures (Mardiastuti, 2001). Phylum Mollusca with more than 100000 recognized species (Feldkamp, 2002) play an important role in ecosystem function for forage of predators in their habitats.

The term molluscs are relatively known compared to other components of the mangrove habitats (Kober, 2004; Mardiastuti, 2001; Smith & Nol, 2000). The Gastropoda with an estimated 75000 to 150000 species are the most diverse class of molluscs in the marine habitats (Strong *et al.*, 2008) such as mangroves (Vermeij, 1973) and terrestrial habitats (Barker, 2001). It has been shown that gastropod assemblages massively contribute to feeding resources of waders within the mangrove ecosystem (Al-Sayed *et al.*, 2008). Although classically the role of mangrove gastropods in nutrient dynamics has been largely overlooked, studies have demonstrated their central ecological role (Fratini *et al.*, 2008).

Mangroves are intertidal vegetation along tropical and subtropical shorelines (Zhang *et al.*, 2007), which have special physiological adaptations to frequently inundate by the tides (Lewis Iii, 2005). These

unique ecosystems provide a large number of biological, ecological, economic, scientific, environmental, aesthetic and ethical values (Mitsch, 2005) including controlling tide level (Varnell *et al.*, 2003) reducing effects of wave and wind energy against shorelines (Miththapala, 2008), stabilizing shorelines (Lee & Shih, 2004). Thus mangroves protect inland structures (Lewis Iii, 2005), support coastal fisheries (Walters *et al.*, 2008), provide diverse habitat to support wildlife communities including a large number of waterbirds, especially waders (Lewis Iii, 2005), and so many other direct and indirect benefits (Gustavson *et al.*, 2009; Zhou *et al.*, 2010).

The total area of mangrove forests in Iran, which covering an area of 12481 ha, is estimated to be less than 0.1% of the total area in the world (Safiari, 2002). These mangroves including only two species of *Avicennia marina* (with the most parts) and *Rhizophora mucronata*, are scattered in the eastern coastal of Oman Sea in Goatr Bay Protected Area within quadrant 25˚ 11′-25˚ 16′N and 61˚ 35′-61˚ 28′E (with 671.53 ha), up to mid of Persian Gulf coastal in the Mel-e-Gonzeh Protected Area in the position of 27˚ 52′ N and 51˚ 35′E (with 22 ha). Each one of the mangrove habitats is widely known as one of the most productive with diverse attributes including a typical fauna (Behrouzi-Rad 1991; Danehkar, 2001a; Mohammadizadeh *et al.*, 2009; Safa, 2006; Zehzad *et al.*, 2002) and are highly important because of their role in the food resources, shelter, nesting and roosting sites for wide range of globally important species (Zahed *et al.*, 2010). It has been estimated that approximately 527 bird species occurred in Iran (Lepage, 2010), as if mangroves in Persian Gulf and Oman Sea hold more than 20% of them.

However there is no qualitatively account of diversity of the molluscs, particularly the gastropods, in rich tropical forests not only in the Hormozgan province (Danehkar, 2001), but also in the Indo-West-Pacific mangrove habitats (Lee, 2008). Moreover, to date no detail studies have been done to observe the seasonal diversity and abundance of molluscs as a food resource especially for waders in mangroves of Hormozgan, Iran. Therefore, the main objective of this study was to describe the mollusc's species diversity and abundance based on four seasons in the Hara Protected Area (HPA) and Gaz and Hara Rivers Delta (GHRD) mangrove forest, south of Iran.

MATERIAL AND METHODS

Study Area: The study areas included Hara Protected Area (HPA) and Gaz River and Hara Rivers Delta (GHRD), which are located within quadrants 26˚ 23′–26˚ 59′ N and 55˚ 32′–55˚ 48′ E, and 26˚30′–26˚50′ N and 57˚00′–57˚40′ E respectively (**Fig. 1**).

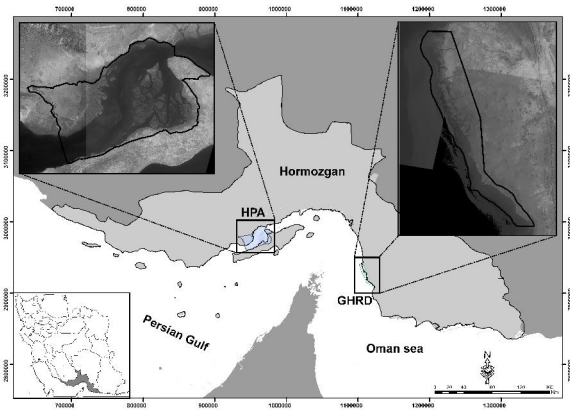

Fig. 1 The location of study areas

Biospheric reserve of Hara Protected Area (HPA) or 'Khouran Straits' is located in the southern Persian Gulf between the region of the Mehran river and Kol river deltas and the island of Qeshm. Within the straits, there are 100,000 ha of low-lying islands, mangrove, mudflats and creeks which constitute much of the largest mangrove/mudflat ecosystem in Iran. The main area of mangrove and mudflat (82360 ha) was designated a Protected Region in 1972. This was later increased to 85,686 ha and upgraded to a National Park (Hara National Park), but downgraded to Protected Area in the 1980s. The entire area is known as 'Khouran Straits' (100000 ha) and was designated a Ramsar Site in 1975, while the reserve (85686 ha) was designated a Biosphere Reserve in 1976 (Danehkar, 1996). The annual mean, minimum and maximum temperatures are 27.6˚C, 2˚C and 48˚C in a 30 years period (1975-2005), respectively. The mean annual rainfall is about 80.3 mm that mainly occurs in the winter. The mean monthly relative humidity is 83.4% and the range of high tide is 4.33 m from the Port of Shahid Rajaee, nearest to the study site. The mangrove species of *Avicennia marina* is the pure stand in this area.

The international wetland of Gaz and Hara Rivers Delta (GHRD), with 15000 ha area, is a large area of intertidal mudflats and mangrove swamps at the mouths of two rivers on the eastern shore of the Straits of Hormoz, at the entrance to the Persian Gulf. The entire wetland has been designated a Ramsar site in 1975 and has been identified as an Important Bird Area by Birdlife International. The minimum, maximum and

annual mean temperatures are 3.5˚C, 49.6˚C and 26.5˚C over a 30 year period (1975-2005) at the Minab meteorological station, respectively. The mean annual rainfall is about 40.6 mm that mainly occurs in the winter. The lowest mean monthly rainfall (0 mm) occurred over 6 months, between April and October. Highest monthly rainfall (19.6 mm) occurred in January. The mean annual relative humidity is 77.9%. The patch of mangrove forest, at the mouth of the rivers, is probably the finest stand of *Rhizophora*, in terms of tree size and density.

Survey Design: Square plot sampling method carried out in its most basic form. Each site was divided by many intertidal channels. A total of 3 transects had been established on the map randomly within three main channels in each area. Transects were run parallel to creek at the pre-decided locations distributed in each area (**Fig. 2**). A total of 35 point count stations (300 m apart from each other) were established within transect 1, 30 points in transect 2 and 32 points in transect 3, randomly in the HPA and similar trends spread on GHRD, which 30 points were established within each transect.

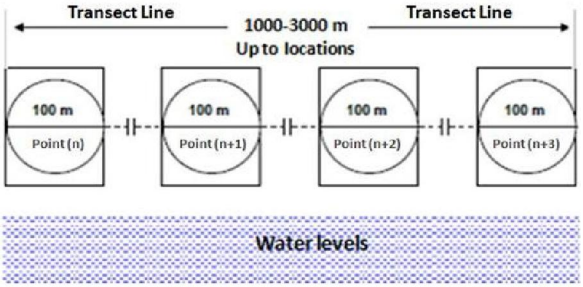

Fig. 1 Randomization of sampling points among transects

Gastropod survey: Overall, the survey was established during four seasons including fall, winter, spring and summer.

All molluscs (gastropods) were collected by hand picking, using 0.0625 square meter quadrates with size of 25 x 25 cm that were chosen randomly within each selected point. At the same time the foulers like mussels and oysters were collected by scrapping those using knives or spatula from the quadrate. Also, gastropod (larger than 0.5 mm) was measured to a depth of 10 cm using special sediment sample dishes (10 cm Diameter). After washing with sea water and sieving the samples, 70% Ethyl alcohol and formalin (4%) was added drop by drop to water in which animals were kept. Then the samples were transferred to the laboratory of Azad University-Bandar Abbas branch. In the laboratory, each sample was washed by fresh water. Gastropod samples were identified using field guides and identification keys (Dance, 1974) and counted.

***Data Analysis*:** Gastropod communities in the studied plots were characterized and counted per each 0.625 square meter plot. The relative abundance (%) of species was determined using the expression: *n/N x 100* (Where *n* is numbers of recorded species and *N* is total observations recorded) (Zakaria *et al.*, 2009).

The mean of parameters (±SE) and one-way analysis of variance (ANOVA) followed by a *post hoc* multiple comparison (Tukey's test) were calculated to compare the mean values of observation based on season.

A X^2 test was applied to look at significant differences between gastropod communities in the *A. marina* and *R. mucronata* habitats. Additionally, a principal components analysis (PCA) was used to determine the level of contribution of species by seasons.

All statistical analyses were also performed with SPSS version 16.0 (SPSS Inc., Chicago, Illinois, USA).

As a noted by Seaby & Henderson (2007), even a quite modest field survey can produce a bewildering amount of information on the presence and abundance of species. As it is commonly difficult to identify the main features and inter-relationships between communities, thus, the similarity percentages (SIMPER), analysis of similarity (ANOSIM) and cluster analysis of communities were tested for comparing and classifying communities using the community analysis package software (CAP version 4.0).

RESULTS

For a period of one year of sampling, started from September 2^{1th} 2008, a total of 1581 individuals of gastropods, representing 28 species and 21 families, were observed in the two sites. Based on the data in each site, a total of 770 gastropods, belonging to 28 species were recorded at the *A. marina* habitat in the mangroves of HPA, while a total of 811 individuals, belonging to 20 species at the *R. mucronata* habitat of GHRD. **Table 1** gives the classification and relative abundance of gastropods according to superfamily and family in the two types of mangrove forests.

The observations were categorized based on different season, where a total of 210 (21 spp.), 336 (23 spp.), 131 (17 spp.) and 93 (12 spp.) observations were

recorded at HPA in the fall, winter, spring and summer, respectively. While, a total of 268 (13 spp.), 248 (20 spp.), 150 (9 spp.) and 145 (8 spp.) observations were recorded at GHRD in the same seasons, respectively. The mean value of gastropod species in HPA during fall, winter, spring and summer seasons were estimated at 7.50±3.22, 12±4.56, 4.68±1.68 and 3.32±1.34 individuals per plot respectively. While, the mean value in GHRD were estimated at 12.75±8.56, 11.81±7.85, 7.14±5.04 and 6.90±5.08 individuals per plot in the same seasons, respectively. Moreover, the results showed that there was a significant difference between the number of individuals observed due to seasons (p<0.01) in both habitats. *Post hoc* multiple comparisons also clearly indicated that more individuals were recorded in winter than in the fall (p<0.05), spring (p<0.05) and summer (p<0.05) in both habitats.

The correlation of various gastropod species and season of winter as presented in the principal component analysis (PCA) plot indicated that most species were correlated with winter excluding four species namely *Ethalia sp.*, *Haminoea sp.*, *Trichotropis sp.* and *Tibia insulaechorab curta* at HPA **(Fig. 3)**, and three species namely *Telescopium telescopium*, *Stocsicia annulata*, and *Stenothyra arabica* at GHRD (**Fig. 4**). The axis 1 and 2 explained 60.88% and 26.64% for HPA and 59.97% and 31.22% for GHRD respectively. The results also showed that the eigenvalues for axes 1, 2, 3 and 4 were 0.819, 0.112, 0.069 and 0.000 respectively, and also cumulative percentage variance of species data for axes 1, 2, 3 and 4 were 81.9, 93.1, 100.0 and 0.0 for HPA respectively, while for GHRD, the eigenvalues for axes 1, 2, 3 and 4 were 0.803, 0.150, 0.048 and 0.000 respectively, and also cumulative percentage variance of species data for axes 1, 2, 3 and 4 were 80.3, 95.2, 100.0 and 0.0 respectively.

Number of individual and species of molluscs at two sites in each season were compared (**Table 2**). Mean value of molluscs' species was estimated at 6.88±2.77 (sp. per plot) versus 9.65±6.63 (sp. per plot) at HPA and GHRD respectively. The results of X^2 test indicated that there was a high significant difference between total gastropod population observed at four seasons ($X^2_{3,\ 1}$=31.9, p<0.001), but there was no significant difference in term of number of species between sites in order to seasonal observation ($X^2_{3,\ 1}$=0.84, p>0.05). Furthermore, diversity comparisons of gastropod assemblages by Rényi diversity profiles (**Fig. 5**) and sample rarefaction (**Fig. 6**) at HPA and GHRD indicated that the highest diversity was at HPA as compared to GHRD.

A similarity percentage (SIMPER) analysis indicated that mangroves of HPA and GHRD were dominated with Asseminea. However, the number of populations was much higher at *R. mucronata* habitat (SIMPER, percentage of contribution to similarity of 37.96 % and 79.15 %, respectively) (**Table 3**). Eight species namely *Asseminea sp.*, *Stenothyra arabica*, *Cerithidium cerithinum*, *Littoria intermedia*, *Telescopium telescopium*, *Iravadia quadrasi*, *Atys cylindrica* and *Cyclostrema ocrinium* represented more than 91% of observations at HPA, while at GHRD, there were only three species namely *Asseminea sp.*, *Stenothyra arabica* and *Cerithidea cingulata* which represented more than 90% of observations. There was no new species in GHRD than HPA. Cluster analysis illustrated that gastropod abundances, fell into two main groups based on season accessions (**Fig. 7).**

The analysis of similarity (ANOSIM) was also performed to test the patterns of the species composition between two habitats. The ANOSIM determined that there was significant difference between composition of gastropod species in the two habitats (p<0.01) (**Table 4**).

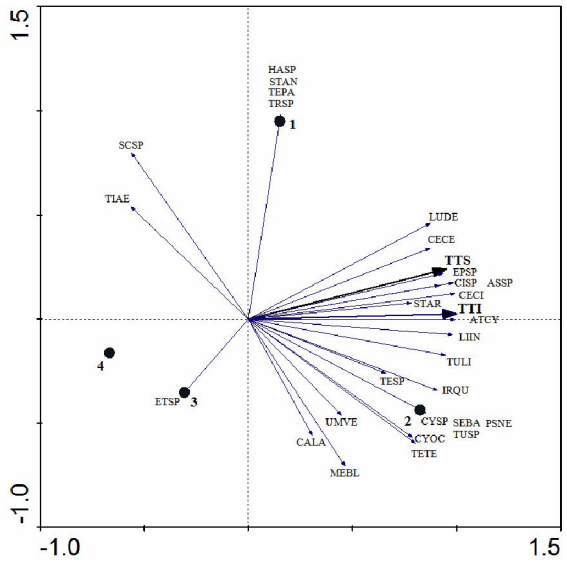

Fig. 3 The correlation of gastropods based on seasons using PCA plot in the HPA

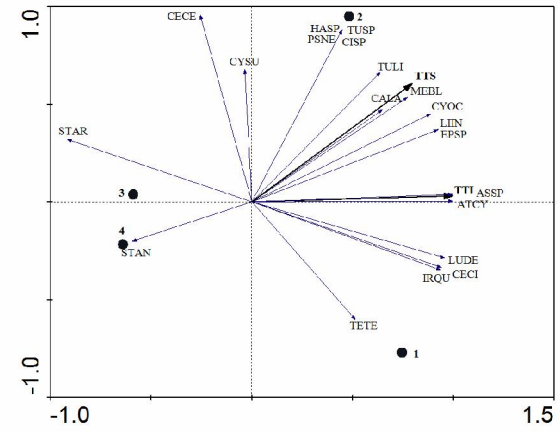

Fig. 4 The correlation of gastropods based on seasons using PCA plot in the GHRD

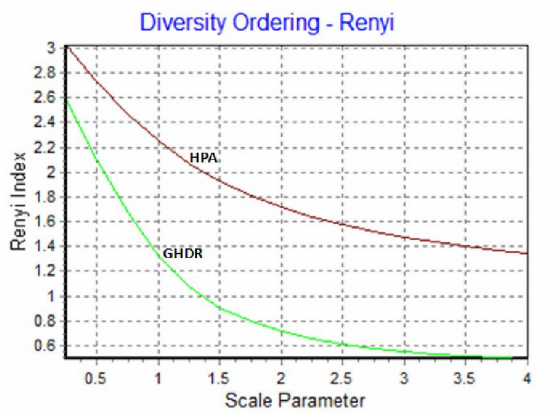

Fig. 5 Diversity comparisons of gastropod assemblages by Rényi diversity profiles at HPA and GHRD

Samples - 1: fall, 2: winter, 3: spring and 4: summer
Species - ASSP: Asseminea sp., ATCY: Atys cylindrica, CALA*: Cassidula labrella*, CECI*: Cerithidea cingulata*, CECE*: Cerithidium cerithinum*, CISP: Citharmagllia sp., CYOC*: Cyclostrema ocrinium*, CYSU*: Cyclostrema supremum*, EPSP: Epithonium sp., HASP: Haminoea sp., IRQU*: Iravadia quadrasi*, LIIN*: Littoria intermedia*, LUDE*: Lucidinella densilabrum*, MEBL*: Mitrella blanda*, PSNE*: Pseudominolia nedyma*, STAR*: Stenothyra arabica*, STAN*: Stocsicia annulata*, TETE*: Telescopium telescopium*, TULI*: Turbonilla linjaica*, TUSP: Turitella sp. , TTI: Total Individual Observations, and TTS: Total Species Observations

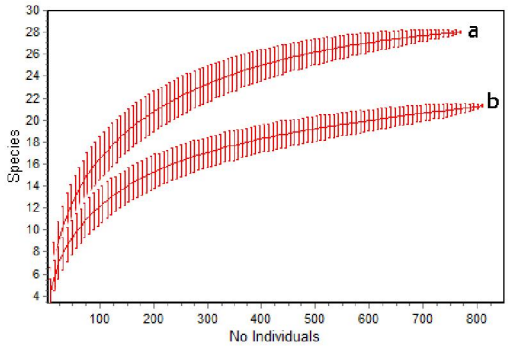

Fig. 6 Rarefaction patterns of gastropods at HPA (6) and GHRD (6)

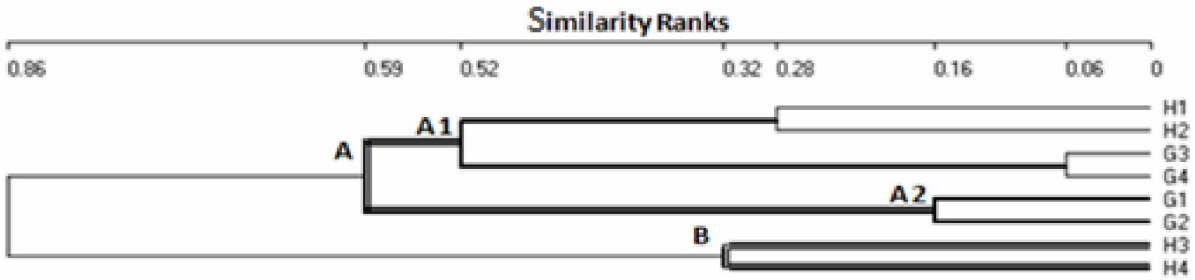

Fig. 7 Grouping of gastropod assemblages as defined by cluster analysis across seasonal changes at HPA (H) and GHRD (G). Note: 1-fall, 2-winter, 3-spring and 4- summer

Table 1 Classification and relative abundance of Gastropods according to Superfamily and family in the HPA and GHRD mangrove forest

Superfamily	Family	Species	Abundance	
			HPA	GHRD
Buccinoidea	Columbellidae *Swainson,1840*	*Mitrella blanda* (Sowerby, 1844)	2	8
Capuloidea	Capulidae *Fleming, 1822*[1]	*Trichotropis sp.*	1	0
Cerithioidea	Diastomatidae *Cossmann, 1894*[2]	*Cerithidium cerithinum* (Phlippi, 1849)	7	5
	Potamididae *Adams & Adams,1854*[3]	*Cerithidea cingulata* (Gmelin, 1791)	90	62
		Telescopium telescopium (Linnacus, 1758)	39	14
		Terebalia palustris (Linnaeus,1767)	2	0
	Turritellidae *Mörch, 1852*	*Turitella sp*	3	1
Conoidea	Turridae *Adams & Adams,1854*	*Citharmagllia sp.*	6	1
	Terebridae *Mörch, 1852*	*Terebra sp.*	7	0
Ellobioidea	Ellobiidae *Pfeiffer,1854*	*Cassidula labrella* (Deschayes,1830)	8	8
Epitonioidea	Epitoniidae *Berry, 1910*	*Epithonium sp.*	3	3
Haminoeoidea	Haminoeidae *Pilsbry, 1895*	*Atys cylindrica* (Helbling,1779)	34	5
		Haminoea sp.	3	1
Littorinoidea	Littorinidae *Chilren,1834*	*Littoria intermedia* (Philippi, 1846)	61	3
Pyramidelloidea	Pyramidellidae *Gray,1840*	*Turbonilla linjaica* (Melvill & Standes, 1901)	6	6
Rissooidea	Assimineidae *Adams & Adams,1856*	*Asseminea sp.*	279	560
	Iravadiidae *Thiele, 1928*	*Iravadia quadrasi* (Boettger,1893)	38	31
		Lucidinella densilabrum (Melvill, 1912)	4	30
	Rissoidae *Gray,1847*	*Stocsicia annulata* (Dunker, 1860)	2	1
	Scaphandridae	*Scaphander sp.*	12	0
	Stenothyridae	*Stenothyra arabica* (Neubert, 1998)	106	55
Stromboidea	Strombidae *Rafinesque, 1815*	*Tibia insulaechorab curta* (Sowerby II, 1842)	9	0
Triphoroidea	Cerithiopsidae *Adams and Adams,1854*	*Selia bandorensis* (Melvill,1893)	1	0
Trochoidea	Turbinidae *Rafinesque, 1815*	*Cyclostrema ocrinium* (Melvill & Standes, 1901)	26	11
Trochoidea	Turbinidae *Rafinesque, 1815*	*Cyclostrema supremum*	2	5
	Trochidae *Rafinesque, 1815*	*Ethalia sp.*	2	0
		Pseudominolia nedyma (Melvill, 1897)	1	1
		Umbonium vestiarium (Linnacus,1958)	16	0

[1]Synonyms: Trichotropidae Gray, 1850; Verenidae Gray, 1857; Pileopsidae Chenu, 1859; Siriidae Iredale, 1931; Cerithhiodermatidae Hacobjan, 1976

[2] Synonyms: Ewekoroiidae Adegoke, 1977

[3] Synonyms: Telescopiidae Allan, 1950; Cerithideidae Houbrick, 1988

Table 2 Number of gastropod species and individuals at HPA and GHDR

Location/Season	No. Species	No. Individuals	Relative Abundance	Mean Value	SE
HPA	**28**	**770**	**100.00**	**6.88**	**2.70**
Fall	21	210	27.27	7.50	3.22
Winter	23	336	43.64	12.0	4.56
Spring	17	131	17.01	4.68	1.68
Summer	12	93	12.08	3.32	1.34
GHRD	**20**	**811**	**100.00**	**9.65**	**6.63**
Fall	13	268	33.05	12.76	8.56
Winter	19	248	30.58	11.81	7.85
Spring	9	150	18.50	7.14	5.04
Summer	8	145	17.88	6.90	5.08

Table 3 Contribution of mollusc's species towards differentiating the types of mangroves habitat (SIMPER Analysis)

Location and Species	Average Abundance	Average Similarity	Contribution (%)	Cumulative (%)
HPA		***60.04***		
Asseminea sp.	69.75	22.79	37.96	37.96
Stenothyra arabica	26.50	10.98	18.30	56.25
Cerithidea cingulata	22.50	6.54	10.89	67.15
Littoria intermedia	15.25	3.79	6.31	73.45
Telescopium telescopium	9.75	3.61	6.02	79.47
Iravadia quadrasi	9.50	2.76	4.60	84.07
Atys cylindrica	8.50	2.74	4.57	88.64
Cyclostrema ocrinium	6.50	1.65	2.75	91.39
GHRD		***72.77***		
Asseminea sp.	140.00	57.60	79.15	79.15
Stenothyra arabica	13.75	4.28	5.88	85.04
Cerithidea cingulata	15.50	4.13	5.67	90.71

Table 4 The ANOSIM pair-wise tests for similarity between sites due to 4 seasons

First Habitat	Second Habitat	Permutations done	P Value	Level %	No >= Obs.	Quadrate Stat.
HPA	GHDR	35	0.01	2.85	1	0.5

DISCUSION

Mangrove forests offer a considerable variety of food resources for many waterbird species (Skilleter & Warren, 2000). The molluscan community is a key component of the mangroves' food chain (Morrisey *et al.*, 2003; Nanami *et al.*, 2005; Okuda & Nishihira, 2002), and play a vital role in the abundance of waders (Al-Sayed *et al.*, 2008; Perez-Hurtado *et al.*, 1997; Piersma *et al.*, 1993) and of some seabirds (Bond & Diamond, 2007; Petry *et al.*, 2008). On the other hand, abundance and diversity of molluscs have been, historically, used as an indicator of ecosystem health and of local biodiversity in mangrove (Amin *et al.*, 2009; Bryan *et al.*, 2009).

The studies dealing with the molluscan community at mangroves areas are insufficient not only in the Hormozgan province (Rohipour, 2007), but also in the Persian Gulf and Oman Sea (Alsharhan & Kendall, 1994; Barth, 2003). The purpose of the present study was to determine the assemblage structure of invertebrates in relation to four seasons to finding the effect of their abundance in the abundance of waterbirds in the mangroves of Hormozgan province.

The structure of the molluscan community present in the HPA and GHRD mangrove forests showed that family of Assimineidae, Potamididae and Stenothyridae were the dominant family. Comparing with similar studies (Danehkar, 1994; Rohipour, 2007; Safa, 2006) conducted in the Hormozgan mangroves the results showed a similarity in molluscan diversity among them, where *Asseminea sp.*, *Stenothyra arabica* and *Cerithidea cingulata* are usually present in the Persian Gulf mangrove areas. The same is also true for Oman Sea. Additionally, the results of this study showed that the diversity of the molluscan fauna in the HPA was higher than in GHRD, however the total abundance in GHRD had also been considered. This can be reflected in the fluctuation of waterbird populations, where the presence of molluscs showed temporal oscillations in order to seasons. It may be related in part to a combination of factors, including temperature, low water flow, the rate of organic matter and subsequent decrease of dissolved oxygen concentration.

The finding of *Terebralia palustris* at HPA is interesting. Since this species was rarely found and have been declining in the world in the recent decades, this finding has ecological and conservational

importance. This large and distinctive edible gastropod was absent in the previous studies (Al-Khayat, 1997; Al-Sayed *et al.*, 2008; Dodd *et al.*, 1999; Rohipour, 2007) in the mangroves of Persian Gulf coast. It is suggested that the reasons for the contemporary absence of *T. palustris* within the Persian Gulf remain speculative. Elsewhere in the Indo-Pacific it is typical (Fratini *et al.*, 2004), although not always (Feulner & Hornby, 2006), closely associated with mangrove forests, so the reduced presence of mangroves in Persian Gulf limits, where they are near the margin of their winter frost tolerance, has been tentatively invoked to explain the absence of *T. palustris* there.

As mentioned in the literature review, despite the lower species richness, abundance of gastropods is generally high. It is related to low degree of environmental favorableness in mangrove habitats, which have remarkable adaptations to changes in environmental conditions and are able to attain high levels of primary productivity. Part of the adaptation, however, is to minimize nutrient drain through organic matter loss. Also, the results showed the highest number of molluscs during waterbird migratory seasons (winter at HPA and fall at GHRD) than non-migratory season and the assemblages are dominated by large populations of few species that are adapted to the environmental limitations.

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Barriers of Local Participation in Rural Cooperatives A Case Study of Fars, Iran

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Abstract: Local participation has an important role in development of rural cooperatives. This article attempts to illustrate the barriers of people participation in rural cooperatives in Fars Province, Iran. Rural cooperatives are certainly a major contributor to rural development in many countries. But, in this case there are a significant number of barriers to effectively using rural cooperatives as a tool for rural development. This paper used qualitative approach to illustrated barriers of cooperatives through local participation. The findings through focus group identified several constraints that have limited active local participation in rural cooperatives.
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Keywords: participation, rural cooperatives, rural development

Introduction

Rural cooperatives have played an important role in the development of agriculture in industrialized countries as suppliers of farming requisites, marketers of agricultural commodities, and providing services such as gain storage and transport. It appears that many of these agricultural cooperatives are adapting their operations to the rapidly changing economic environment characterized by technological change, industrialization of agriculture and growing individualism (Ortmann & King, 2007).

The rural cooperatives in most developing countries are faced with constraints with regard to achieving the goal of rural sustainable development, which promotes social and economic development of local communities. In developing countries attempts to organize farmers into rural cooperatives have often failed, although rural cooperatives have the potential to supply farm inputs and market farm products that are both important for agricultural development (Hoyt, 1989). Rural cooperatives are generally considered as a tool for rural development. Many developed countries such an England, France, German and United Stated largely depend on incomes earned through rural cooperatives. It is the intention of this article to bring to discussion the constraints of people participation towards rural cooperatives development. The rural cooperatives in Iran in the recent years have diversified themselves into various areas of socio-economic activities. The failure of the government sector and various limitations of the private sector have compelled the policy-makers to pin their faiths on the cooperative system (Aref & Sarjit, 2009). Hence, this paper attempted to outline the concept of rural cooperatives and its limitation towards rural development.

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Literature review

People are generally motivated to form co-operatives to obtain or provide goods and services to themselves or to the general community. In the process of providing such goods and services, the founding members, also own and control the operations and processes in a manner acceptable to the majority of the other members. Some of the benefits of co-operatives have been researched and published in various academic journals (Wickremarachchi, 2003).

A cooperative is defined by the International Cooperative Alliance (ICA), as a group of people who join together voluntarily to meet their common economic, social, and cultural needs and aspirations in accord with the following principles: open and voluntary membership, democratic member control, autonomy and independence, member economic participation, member training and education, cooperation among cooperatives, and concern for community. Co-operatives are also based on the values of self-help, self-responsibility, democracy, equality, equity and solidarity. In the tradition of their founders, co-operative members believe in the ethical values of honesty, openness, social responsibility and caring for others (chloupkova et al., 2003). Many theoretical papers (Bendrick & Egan, 1995; Brennan & Luloff, 2005) and a few studies (Bateman et al., 2005; Chloupkova et al., 2003; Majee & Hoyt, 2009; Saegert & Winkel, 1998) have made the case that

cooperatives are well placed to help poor people in strengthening their participation and inclusion in economic activities at the community level (Wilson Majee & Hoyt, 2010).

The International Cooperative Alliance (ICA, 2005) defines a cooperative as "an autonomous association of persons united voluntarily to meet their common economic, social, and cultural needs and aspirations through a jointly-owned and democratically-controlled enterprise". The seven internationally recognized cooperative principles are: voluntary and open membership; democratic member control; member economic participation; autonomy and independence; provision of education, training and information; cooperation among cooperatives; and concern for the community (Ortmann & King, 2007). Rural cooperatives are now considered strong vehicles for community empowerment, which is a big paradigm shift. As compared to the past, the cooperatives by empowering the people have helped eliminate poverty, sustain employment, enrich social standards, and provide employment. Empowerment can be of different levels. Cooperatives are the organizations, which are deeply embedded to the communities in which they serve. Cooperatives provide a strong democratic medium to empower the people (Verma, 2008). DeFourney (1992) argues that co-operatives have assets and qualities in areas, which cannot be claimed by other types of organizations. These include:

• Self-initiative,
• Sensitivity to local needs,
• Reducing the need for public regulation

In terms of rural cooperatives and rural development; the notion of participation has main impact on development of cooperatives. The concept of participation has been traced as far back as the 1950s in development discourse. A fundamental shift in development thinking according to Wainwright and Wehrmeyer (1998) occurred from the technology-dominated paradigm developed in the 1960s toward a more people-centered approach of sustainable growth that saw the emergence of participation in development activities. Participation like 'development' is viewed as having the capacity to bring about positive change and something which everyone has come to believe in and support. Participation has been viewed as a process that leads to empowerment. It can therefore be argued that the ultimate goal of participation is empowerment. Participation then is about power relations.

The rural cooperative has worked in Iran After land reform in 1963. Today there are more than 10000 rural cooperative. However, their ability of these cooperatives is limited and the Iran government is still not doing considering cooperatives as one of the development factors for rural area (Fariborz Aref & Sarjit, 2009).

Research design

The objective of this paper is to present the problems associated with rural cooperatives. Focus group discussions (FGD) were considered as an effective technique to generate information on rural cooperatives since it involves a range of stakeholders. For instance, Bedford and Burgess (2001, 124) are of the view that 'focus groups are especially useful when you want to compare the 'world views' of different sectors or groups of people in an efficient way.'

A total of 110 members from the cooperative comprising both the men and women were present. In this study the general purpose is to investigate the barriers of agriculture development in Fars' villages in Iran. Fars is one of the 30 provinces and known as cultural capital of Iran. It is in the south of the country and its center is Shiraz. It has an area of 122,400 km. In 2006, this province had a population of 4.34 million people, of which 61.2% were registered as urban dwellers, 38.1% villagers, and 0.7% nomad tribes. Agriculture is of great importance in Fars. The major products include cereal, citrus fruits, dates, sugar beets and cotton (Wikipedia, 2010). Iranian agriculture is thousands of years old and this reflects the length of time during which soil and water resources of the country have been utilized for crop production. This study is based on quantitative methodology to investigate the barriers of participation in rural cooperatives. Hence to achieve the objectives of this study, the researcher uses quantitative method.

Twenty two villages in Fars Province, Iran were selected as a case study area because it provided many opportunities to develop rural agriculture. Focus group discussion (FGD) was performed to collection data from local farmers. FGD was conducted in a group setting and was used for obtaining a better understanding of participants' attitudes towards the barriers of agriculture development. There is no consensus among qualitative researchers on the optimal number of participants in FGD. But the ideal number of participants in each FGD is six to ten. Participants of FGD were classified according to their place in the villages. All respondents were male. They ranged in age from 23 to 77 years. The researcher explained to

them the objectives of the study and what questions would be asked. The researchers examined, categorized participants responses from each focus group of villagers that were recorded in video tapes.

Result & Discussion

Findings have shown that rural people are reluctant to get involved in agricultural activities for lack of interest. The lack of effective collaboration and participation in planning and management is rural area.

Lack of local capacity building

Based on the FGD, members in the group expressed concern on the inadequate training sessions. The focus group members argued lack of capacity building is a main reason to lack of local participation for rural cooperatives. The lack of sufficient knowledge and training can be argued as one factor hindering most locals from taking up the responsibility. Building capacity within the community to manage the resources can enhance effective devolution at local levels. The community can also have an opportunity to participate fully in ecotourism activities in the area. Findings also illustrated that the rural cooperative structure dependence on the government. Hence, there is little capacity building with local communities

Limited support at community level

The FGD groups believed local participation that leads to community empowerment for local people is one of the major characteristics of the rural cooperatives. Therefore, the study found that legal rights to aid active community participation have not be been fully devolved at local level. The role of the community to be active participants in cooperatives management is lost. In fact, based on FGD dependency on government and lack of authority in communities was the main problem for development of rural cooperatives.

Insufficient incentives to participate

Insufficient incentive to participate in rural cooperatives was other barrier which has been discussed by participant in FGD groups. Incentive is now argued as essential to further the enhancement of community participation in rural cooperatives. It is argued that 'incentives motivate communities and other role players to not only participate in development of rural cooperatives, but also to manage natural resources sustainably' (Fabricius 2004). However, the challenge is to what extent the majority of the community members are able to access these incentives in order for them to support rural cooperatives.

Insufficient financial support

The lack of financial funds is one major factor that hinders development of rural cooperatives. As has been identified, revenue from the agriculture activities has not been sufficient to meet community activities. On the other hand, government donor funds have not been sustainable. Members of a focus group also identified the lack of funds as one major factor that has limited development of their community cooperatives. According to respondents some locals have not met these requirements and have not been able to access government donor funds.

Lack of local partnerships

According to FGD a general problem for participation in rural cooperatives is the lack of lack of local partnerships. Another major constraint identified by the study is the absence of business partnerships with the local cooperatives. It has been suggested that local communities need partnerships to succeed in such local ventures for them to increase on their income sources. However, many authorities seem unconvinced of the desirability of building true partnerships with the communities and still view rural communities as technically unable and politically underprepared to play a serious role in extension of cooperatives (Barrow & Fabricius, 2002).

Based on the finding of this study, the barriers of rural cooperatives in Fars province can be categorized in three levels:

2-Barriers at the individual level: These barriers include the lack of skill and knowledge, insufficient incentives to participate and lack of empowerment among individuals.

3-Barriers at the organizational level: These barriers are usually associated with rural organization, lack insufficient financial support and lack of capable rural cooperatives leaders.

3-Barriers at the community level: These barriers are related to community factors, which include limited participation, lack of local partnerships, limited support at community level, lack of rural empowerment in cooperatives decision-making and lack of appropriate rural structure.

Conclusion

This paper has identified the barriers of local participation in rural cooperatives development.

Rural cooperatives have played an important role in the development of agriculture in industrialized countries (Aref, 2011). Beside this potential, rural cooperatives in most Third World countries are faced with some barriers. Lack of community resources and capacity building were an important element contributing to limited rural cooperatives development. They refereed to government policy and lack of local capacity as main barriers related rural cooperatives. Clearly, the described barriers may not be only specific to Fars province; some of them may also be considered as common general problems of rural cooperatives development in other communities in Iran. Base on the findings, it can be suggest that rural empowerment can be a tool for development of rural agriculture in Iran. An understanding of the existing barriers of rural participation provides basic information for setting a policy agenda to enhance rural agriculture. Further, it is important for government to understand that rural also face barriers that can hinder its progress in responding and recognizing the priorities of local communities in Iran.

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Biofilm Formation by Blood Stream Staphylococcal Isolates from Febrile Pediatric Cancer Patients at South Egypt Cancer Institute

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Abstract: Blood stream infection (BSI) remains the major cause of morbidity and death in patients undergoing treatment for cancer. Approximately 10% to 30% of all febrile neutropenic cancer patients are bacteremic at presentation. Staphylococci are the most frequently isolated organisms from blood cultures of febrile neutropenic (FN) cancer patients.

Aims: This study aimed to define the main causative organisms of 139 episodes of bacteremia in 100 febrile neutropenic pediatric patients admitted to South Egypt Cancer Institute (SECI), pediatric oncology ward. Also to study the prevalence of biofilm forming capability of the coagulase-negative staphylococci (CONS) and *Staphylococcus aureus* (*S. aureus*) blood isolates (n=36, group A) and in 29 staphylococcal strains isolated from skin and nasal mucosa of healthy care workers (group B).

Methods: All Isolates were identified and tested for antibiotic susceptibility by MicroScan WalkAway System. The CONS and *S. aureus* isolates from blood cultures of pediatric patients were then tested for slime production using qualitative congo red agar plate test (CRA test), quantitative microtitre plate assay (MTP). The presence of *icaA* and *icaD* genes by polymerase chain reaction (PCR) was also determined.

Results: Among 139 episodes of fever and neutropenia recorded in 100 patients, bacteremia represented 54.7% in which Gram negative organisms constituted 52.6 % from the total episodes obtained and Gram positive staphylococcal isolates were 47.4%. *S. aureus* were 14 strains and CONS were 22 strains. Of the 14 *S. aureus*, 10 strains were *icaA* and *icaD* positive versus 8 strains were CRA test positive and also were MTP positive. Two strains of *S. aureus* were PCR positive for *ica* genes and slime negative on CRA and MTP. Of the 22 CONS, 12 (53%) were *ica* genes positive versus 11 strains (46%) were positive using CRA test and 9 strains were MTP positive. One strain of CONS was positive using MTP and PCR negative. Group B isolates were CRA, MTP and *ica* genes negative. Biofilm forming staphylococcal strains on CRA (15/19) and (16/22) with *ica* genes were resistant to imipenem, amoxicillin/clavulanic, cephlosporins, and oxacillin.

Conclusions: The present study shows a high percent of Gram negative bacteremia in pediatric oncology ward and the isolates expressing *ica* genes were exhibiting more resistance to broad spectrum antibiotics. This supports that biofilm adds to the virulence profile of staphylococci isolated from blood stream infections and that the *ica* genes are important virulence markers for clinically significant CONS isolates. The better agreement between the CRA plate tests with the molecular detection of *ica* genes indicates the former as a reliable test for the phenotypic characterization of virulence of clinical isolates.

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1. Introduction:

Blood stream infections (BSI) remains the main cause of morbidity and death in patients undergoing treatment for cancer. Approximately 10%-30% of all febrile neutropenic cancer patients are bacteremic at presentation. Pediatric cancer patients are more susceptible to infections especially in patients with haematological malignancy (Lee *et al*., 1990; Rahiala *et al*., 1998). Cancer patients are predisposed to BSI due to changes in both cell mediated and humoral immunity that is related to primary tumour and subsequent treatment. Therefore, pediatric cancer patients need to be treated early and promptly with proper antibiotics until the blood culture results are available. Cancer patients are more susceptible to infection associated with health care because of their compromised immune system, use of invasive technologies, surgical operations and chemotherapy. Institutions that provide care for cancer patients are expected to have higher rates of nosocomial infections than general care hospitals (Kelly *et al.*, 2010).

There is a shift of microbial spectrum of cancer patients from Gram negative to Gram positive species. Factors that contribute to this shift may be intensive chemotherapy that leads to damage of mucosal barriers, that increases the risk of infection with oral and gastrointestinal flora. In addition the use of implantable intravenous catheters can facilitate entry of organisms colonizing the skin into the blood stream and thus increase the rate of staphylococcal infections (Viscoli *et al*., 1999).

The production of capsular polysaccharides by coagulase negative staphylococci is considered as a virulence factor. Staphylococcal biofilm is mediated by the polysaccharide adhesion (PIA), and referred to product of the *icaABCD* gene cluster that encodes for the N-acetyl glucosaminyl transferase enzyme. N-acetyl glucosaminyl transferase enzyme catalyzes the synthesis of the capsular polysaccharide (b-1,6-glucosamineglycan) from N-acetyl glucosamine. Ziebuhr *et al*. (1997, 1999) detected the *ica* locus in 85% of coagulase-negative staphylococci causing invasive infections, but only in 6% of contaminating strains, and proposed targeting the *ica*-locus as a diagnostic marker for pathogenicity in staphylococci.

The capacity of both *S. epidermidis* and *S. aureus* to form biofilm is an important virulence factor in the development of device related infections. This represents a serious clinical problem, given that the majority of hospitalized patients undergo procedures for the insertion of foreign devices, from catheters to artificial heart valves, etc. Moreover, patients susceptible to device-related infections are often colonized with hospital-acquired, multiple antibiotic- resistant organisms and may be further compromised by serious underlying disease or trauma. Significantly, the majority of biofilm-mediated device-related infections are caused by either *S. epidermidis* or *S. aureus* (O'Gara & Humphreys, 2001).

The aims of this study were to monitor the prevalent aerobic microorganisms causing bacteremia in the pediatric oncology department at the South Egypt Cancer Institute (SECI). Also to determine the antimicrobial susceptibility of bacterial isolates and the isolated staphylococci were examined for biofilm production by using qualitative congo red agar plate test (CRA test) and quantitative microtitre plate assay (MTP). The presence of *icaA* and *icaD* genes was determined by polymerase chain reaction. Also to examine biofilm formation by skin and nasal staphylococcal isolates from healthy care workers at the pediatric oncology ward and to assess the relationship between biofilm production and pathogenicity of staphylococci.

2. Patients and Methods

This prospective study was carried out in Pediatrics Oncology Department, at SECI and the Medical Microbiology & Immunology Department, Faculty of Medicine Assuit University during the period from January 2008 to December 2009.

Group (A) included 100 pediatric cancer patients in South Egypt Cancer Institute. Out of 139 fever episodes; number of episodes in males was 86 and in females was 53 and their age ranged between 1-12 years with mean of 6.73± 2.028 years. Full medical history and complete physical examination were performed in search for any septic focus. The study was approved by the Institutional Ethical Committee and patients' consents were obtained from the parents before collection of specimens. The data collected included age, diagnosis, type of chemotherapy, surgery, absolute neutropenic count (ANC) (patients having ANC raised above 500 X 10^9/L were not excluded from the study), grade of fever, ICU admission and type of empirical antibiotic therapy. Exclusion criteria were fever due to chemotherapy and patients under empirical antibiotic therapy. Blood cultures were obtained from group A.
Group (B) included 25 healthy volunteers from healthcare members at the Pediatrics Oncology Department, at SECI as a control group for studying biofilm formation in staphylococci skin and nasal isolates taken from group B.

Two to five milliliters of peripheral blood were aseptically collected from neonates and children up to 10 years of age and 10 ml from children more than 10 years and inoculated into blood culture bottles. Blood cultures (Oxoid) were transported immediately to the Microbiology Laboratory. Blood agar, nutrient agar, mannitol salt agar and MacCkoncy's agar were used for isolation of the organisms. Staphylococcal isolates were preserved in tryptic soya broth with glycerol (15% v/v) at -80°C. Identification of the organisms and antibiotic susceptibility testing was carried via MicroScan WalkAway system 96 (Dade Behring Inc., MicroScan Inc., West Sacramento, CA95691, USA) which is a conventional overnight incubation system that uses the reference broth microdilution method.

Phenotypic characterization of biofilm formation: Congo Red Test:

Congo red test was performed as previously described by Freeman *et al*. (1989) in triplicate and results were interpreted by two different investigators. The medium composed of brain heart infusion broth (37 gm/l), sucrose (5 gm/l), agar (10 gm/l) and congo red dye (0.8 gm/l). Congo red was prepared as concentrated aqueous solution and autoclaved at 121°C for 15 minutes separately from

other medium constituents and was then added when the agar had cooled to 55°C. Plates were inoculated with test organism and incubated at 37°C for 24-48 hours aerobically. On CRA, biofilm-producing strains form black colonies with a dry crystalline consistency, while biofilm non producing strains form pink colonies (Arciola *et. al.*, 2001).

Microtitre Plate Test:

Quantitative determination of biofilm production was carried out as described by Aricola *et al*. (2002). Briefly, overnight grown bacteria in trypticase soya broth (TSB) were diluted (1:100) and 200 ml portions were inoculated into sterile 96-well flat bottom polystyrene microtiter plates. Incubation was carried out at 35°C for 22-24 h before removal of the cultures. The wells were washed 3 times with phosphate buffered saline (PBS, pH, 7.2), air dried and stained with 0.25% crystal violet for 1 min. The optical density of the wells was measured at 570 nm using micro ELISA auto reader (Stat Fax – 2100, AWARENESS Technology Inc.). An optical density of 0.240 was chosen to distinguish biofilm producers from those that did not form biofilm. Biofilm positive and negative strains of *S. epidermidis* were included in each plate as was a negative control of medium without bacteria. The tests were carried out in quadruplicate and all strains were tested on at least two different days. Strains that had given reading values of more than 0.240 were considered strong biofilm forming, Strains that had given readings values more than 0.120 and less than 0.240 were considered weak biofilm forming strains. While strains that had given reading values of less than 0.120 were considered non-biofilm forming strains (Arciola *et al.*, 2002).

PCR for the detection of ica*A* and ica*D* genes:

Bacterial DNA was extracted from a staphylococcal pure colonies grown on blood agar and suspended in nutrient broth using QIAamp Mini DNA extraction kit (QIAGEN Incorporation) according to the manufacturer's instructions. PCR for *icaA* and *icaD* genes was performed using the method described by Arciola *et al*. (2001 a). For the detection of *icaA* gene 5-TCTCTTGCAGGAGCAATCAA-3 was used as the forward primer (corresponding to nucleotides 4796–4815) and 5-TCAGGCACTAACATCCAGCA-3 was used as the reverse primer (corresponding to nucleotides 4964-4983). For *icaD*, 5-ATGGTCAAGCCCAGACAGAG-3 was used as the forward primer (corresponding to nucleotides 5422–5441), and 5-CGTGTTTTCAACATTTAATGCAA-3 was used as the reverse primer (corresponding to nucleotides 5616–5597). Reaction mixtures (50 µl) contained 25 µl PCR master mixture, 1 µl of each primer (0.1-0.5 µM final concentration), 18 µl RNA ase free water & 5 µl of template DNA. Amplifications were performed with the following thermal cycling profile an initial denaturation at 94°C for 2 min., followed by 30 cycles of amplification (denaturation at 94°C for 1 min., primer annealing at 60°C for 1 min., and extension at 72°C for 2 min.) and a final extension for 4 minutes. Amplicons for *icaA* and *icaD* produced fragments of 188 and 198 bp, respectively. The amplified product sizes were estimated by comparison with 100 bp DNA ladder (QIAGEN Incorporation).

Statistical Analysis:

Statistical analyses were conducted using the SPSS package version 17. Mean values and standard deviation were used for data description. Differences in the distribution of individual parameters among patient subsets were analyzed using the T test for categorized variables and Pearson chi-square was used to measure the concordance between *ica* genes and CRA positivity. *P*-value was two-tailed and was considered significant at a level of ≤0.05.

3. Results

During a two years period extending from Jan 2008 to Dec. 2009, Group A: were 100 pediatric cancer patients who suffered from 139 febrile episodes at the SECI. They were 86 males and 53 females with a mean age ± 6.73 ± 2.03 years. Group B: were 25 healthy care volunteers as a control. Table (1) summarizes the characteristics of the pediatric cancer patients involved in this study regarding tumor type, presence or absence of neutropnia, ICU admission or surgical intervention. All patients were using intravenous catheter and were treated empirically by third generation cephalosporins and amikacin.

Bacteriology:

In group A: out of the 139 febrile episodes, 63 blood cultures were negative (45.3%) and 76 blood cultures were positive (54.7%). Gram negative bacteremia represented 52.6% with 40 isolates in which 11 were (14.5%) *Klebsiella*, 8 (10.5%) *Proteus*, 7 (9.2%) *E- Coli*, 3 (3.9%) *Enterobacter*, 4 (5.3%) *Yersenia pseudotuberculosis*, 4 (5.3%) *Shigella* and 2 (2.6%) *Salmonella.*

Gram positive bacteraemia represented 47.4% with 36 Staphylococcal isolates. *S. aureus* were 14 strain (18.5%) and coagulase negative staphylocooci (CONS) were 22 (28.9%) isolates. CONS were distributed as 9 (11.9%) *S. epidermidis*,

5 (6.6%) *S. hominis*, 3 (3.9%) *S. simulans*, 2 (2.6%) *S. haemolyticus*, 2 (2.6%) *S. warneri* and 1 (1.3%) *S. xylosus*.

Group B: 29 Staphylococcal isolates were collected in which 12 were nasal isolates and 18 were skin isolates. They were 8 *S. aureus* and 21 CONS. Table (2) shows number, percent and species identification of isolates collected from blood cultures from febrile pediatric cancer patients using MicroScan WalkAway System.

Antimicrobial susceptibility patterns for *Staphylococci aureus* isolates exhibited 92.8% susceptibility to ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin, gatifloxacin, 85.7% to azithromycin, gentamycin, tetracycline and trimethoprim. However high resistance pattern 71.4% was recorded in amoxicillin/clavulanic acid, ampicillin/sulbactam, cefazolin, cefepime, cefotaxime, cefotriaxone, cephalothin, imipenem and oxacillin. Vancomycin resistance was detected in 42.8% (6/14) of the isolates. Similarly coagulase negative staphylococci exhibited 54.5% resistance to amoxicillin/clavulanic acid, ampicillin/sulbactam, cefazolin, cefepime, cefotaxime, cefotriaxone, cephalothin, imipenem and oxacillin. CONS were still 90.9% susceptible to vancomycin, synercid, rifampin, gatifloxacin, motifloxacin and 86.4% to levofloxacin, and clindamycin.

Among gram negative bacilli, 9/11 *Klebssiella* species were resistant to cefotazidime, ticracillin and to azotrenem, 8/11 were resistant to cefoperazone, cefotizoxime and piperacillin. Highest resistance pattern was contributed with azotrenem (78%) followed by cefotaxime (73%). The least resistance pattern for 40 Gram negative isolates was recorded with amikacin (5/40, 12.5%) and imipenem (7/40, 17.5%).

Biofilm formation:

Biofilm production assessed by CRA reveled that 8/14 (57%) strains of *S. aureus* were biofilm positive and 11/22 strains (50%) of CONS were biofilm forming. All strains of the control group were non-biofilm forming. Quantitative biofilm production determined by microtiter plates assay (MTP) showed that 8 (57%) strains of *S. aureus* were biofilm producers, 7 (50%) strains were strong biofilm producers with readings > 0.240, one (7%) strain was weak biofilm producer, with readings > 0.120 and <0.240. and 6 (43%) strains were non-biofilm producers with readings >0.120. In CONS, 9 (41%) strains were biofilm forming (6 (27%) strains, were strong biofilm producers, and 3 (14%) was weak biofilm producers) and 13 (59%) strains were non-biofilm producers (Fig. 1).

The results of these two phenotypic tests showed that two strains of CONS that had been proved to be biofilm forming with the CRA test appeared to be non-biofilm forming with the spectrophotometer detection. All strains of the control group were non-biofilm forming with OD ≤ 0.120.

Out of 22 CONS strains, 12 strains (53%) show the presence *icaA* gene at 188-bp and 10 strains (46%) were negative for *icaA* gene, while out of 14 *S. aureus* strains, 10 strains (70%) show the presence *icaA* gene and 4 strains (30%) were negative for *icaA* gene (Fig. 2).

The results of *icaA* gene at 188-bp were the same when testing the *icaD* gene amplification product at 198- bp. Staphylococcal strains of group B were negative for both *icaA and icaD* genes.

Table (3) presents comparison between the results from the genotypic testing of biofilm presenting genes *icaA & icaD* via PCR versus those results revealed via phenotypic biofilm CRA and MTP assay. There was 10 strains of *S. aureus icaA* & *icaD* positive versus 8 strains CRA positive & 8 strains MTP positive. Similarly, 12 strains of CONS were *icaA* & *icaD* positive versus 11 strains CRA positive & 9 strains MTP positive. On the other hand there was one strain of *S. epidermidis* positive biofilm on MTP with negative *icaA* and *icaD* gene on PCR. Considering biofilm slime on CRA as gold standard; sensitivity of MTP was 89.5% and specificity was 100%. Taking biofilm slime on CRA as gold standard; sensitivity of PCR was 94.7% and specificity was 76.5%.

Figure (3) demonstrates the relation between biofilm formation and antimicrobial susceptibility pattern showed a higher percent of resistance in biofilm positive isolates i.e.; 15 strains out of 19 biofilm positive strains were resistant to imipenem on the other hand only 7 out of 17 biofilm negative strains were resistant to imipenem. Table (4) shows the clinical characteristics of *ica* gene positive cases compared to those who were negative. No significant differences were encountered between type of tumour, neutropenia or ICU admission among *ica* positive and negative cases.

Table 1:Characteristics of the 139 febrile episodes in 100 pediatric cancer patients.

Characteristics	(N)	(%)
Sex		
Male	86	61.9
Female	53	38.1
Tumour type (Type of chemotherapy)		
ALL (MTX high dose)	51	36.7
AML (ADR + AraC)	36	25.9
NHL (COPADAM)	20	14.4
Wilms tumor (SIOP)	8	5.8
Bone tumors (Platinum based)	3	2.2
Rhabdomyosarcoma (VAC)	5	3.6
Germ cell tumors (PEP)	3	2.2
Neuroblastoma (OPEC-OJEC)	13	9.4
Surgery		
Yes	36	25.9
No	103	74.1
Neutropenia		
Yes	53	38.1
No	29	20.9
Profound	57	41
IV Catheter		
Yes	139	100
Grade of Fever		
Low	48	34.5
High	91	65.5
Empirical Therapy		
3rd Generation + Amikacin	139	100
ICU Admission		
Yes	87	62.6
No	52	37.4

Table 2: Isolated organisms of bacteremia in pediatric cancer patients.

Organisms	(N)	(%)
Gram negative	**40**	**52.6%**
Klebsiella pneumonia	10	13.2
Klebsiella ornithinolytica	1	1.3
Proteus penneri	8	10.5
E. coli	7	9.2
Enterobacter cloaca	3	3.9
Enterobacter aerogenes	1	1.3
Yersinia pseudotuberculosis	4	5.3
Shigella dysenteria	4	5.3
Salmonella Arizona	2	2.6
Gram positive	**36**	**47.4%**
Staphylococcus aureus	14	18.5
Staphylococcus epidermidis	9	11.9
Staphylococcus hominis	5	6.6
Staphylococcus simulans	3	3.9
Staphylococcus haemolyticus	2	2.6
Staphylococcus warneri	2	2.6
Staphylococcus xylosus	1	1.3
Total	**76**	**100%**

Table 3: Biofilm formation in blood stream *Staphylococcal* strain when tested on CRA, MTP and PCR.

		S. aureus	*S. epidermidis*	*S. hominis*	*S. simulans*	*S. haemolyticus*	*S. warneri*	*S. xylosus*	Total
CRA	**Positive**	8	5	4	1	0	1	0	**19**
	Negative	6	4	1	2	2	1	1	17
MTP	**Strong**	7	3	2	1	0	0	0	**13**
	Negative	6	4	2	2	2	2	1	19
	Weak	1	2	1	0	0	0	0	**4**
icaA gene	**Positive**	10	4	4	1	1	1	1	**22**
	Negative	4	5	1	2	1	1	0	14
icaD gene	**Positive**	10	4	4	1	1	1	1	**22**
	Negative	4	5	1	2	1	1	0	14

CRA, congo red agar, MTP, microtitre plate, PCR, polymerase chain reaction

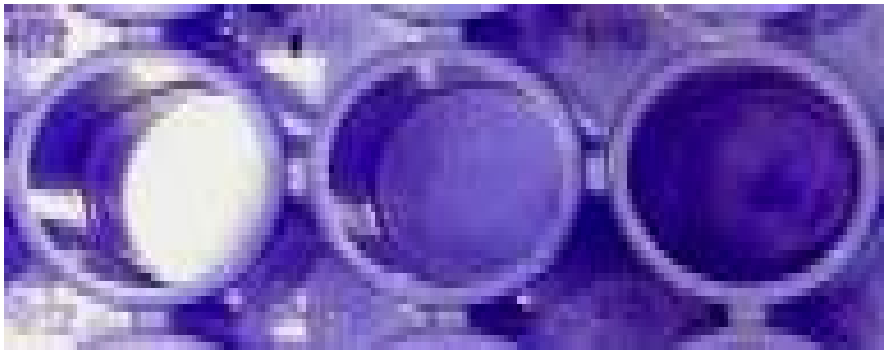
Table 4: The relation between biofilm formation and antimicrobial resistance pattern.

	Biofilm CRA		Biofilm MTP			*ica* genes	
	Positive (19)	Negative (17)	Strong (12)	Week (4)	Negative (20)	Positive (22)	Negative (14)
Amox/K Clav	15	7	11	1	10	16	6
Amp/ Sulbactam	15	7	11	1	10	16	6
Azithromycin	8	2	7	1	2	8	2
Cefazolin	15	7	11	1	10	16	6
Cefepime	15	7	11	1	10	16	6
Cefotaxime	15	7	11	1	10	16	6
Ceftriaxone	15	7	11	1	10	16	6
Cephalothin	15	7	11	1	10	16	6
Chloramphenicol	4	1	3	1	1	4	1
Ciprofloxacin	4	1	4	0	1	4	1
Clindamycin	8	4	6	0	6	9	3
Erythromycin	8	2	7	1	2	8	2
Gatifloxacin	2	1	2	0	1	2	1
Gentamicin	8	3	6	1	4	7	4
Imipenem	15	7	11	1	10	16	6
Levofloxacin	3	1	3	0	1	3	1
Moxifloxacin	2	1	2	0	1	2	1
Ofloxacin	4	1	4	0	1	4	1
Oxacillin	15	7	11	1	10	16	6
Rifampin	4	4	3	0	5	6	2
Synercid	4	4	3	0	5	6	2
Tetracycline	6	3	5	1	3	7	2
Trimeth/Sulfa	7	3	6	1	3	8	2
Vancomycin	4	4	3	0	5	6	2

CRA, congo red agar, MTP, microtitre plate

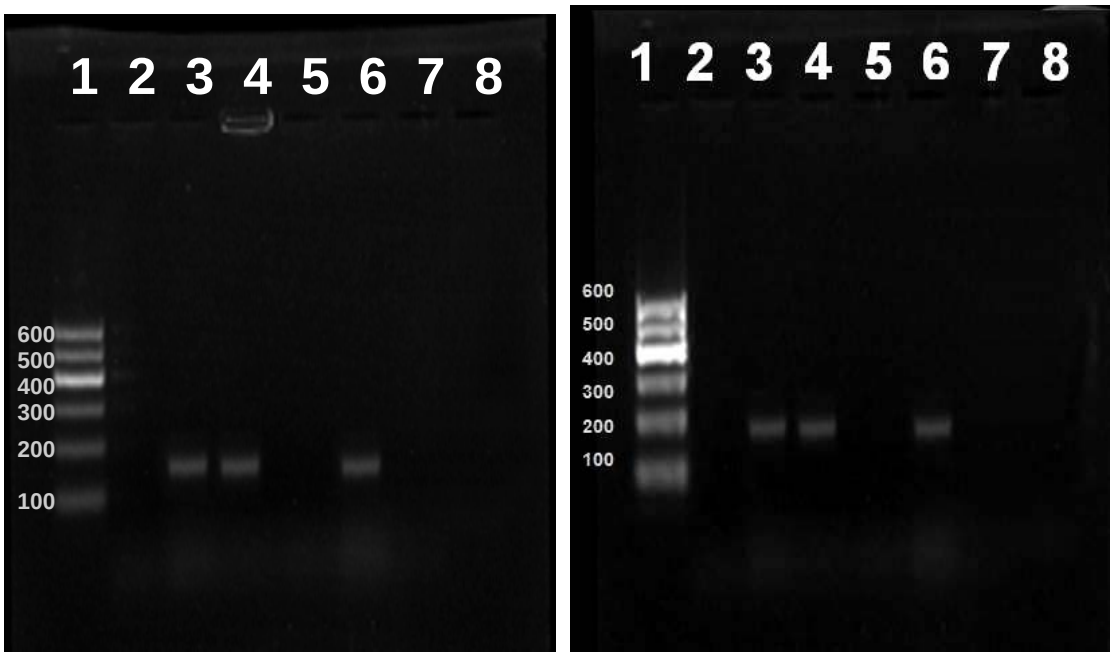
Table 5: Clinical characteristics and *ica* gene.

		ica genes		*P*-value
		Positive N (%)	Negative N (%)	
Sex	Male	11 (47.8)	9 (69.2)	0.21
	Female	12 (52.2)	4 (30.8)	
Tumor type	ALL (MTX high dose)	4 (17.4)	7 (53.8)	0.18
	AML (ADR + AraC)	8 (34.8)	1 (7.7)	
	NHL (COPADAM)	5 (21.7)	3 (23.1)	
	Wilms tumor (SIOP)	3 (13.)	1 (7.7)	
	Rhabdomyosarcoma (VAC)	2 (8.7)	0 (0)	
	Neuroblastoma (OPEC-OJEC)	1 (4.3)	1 (7.7)	
Surgery	Yes	10 (43.5)	4 (30.8)	0.45
	No	13 (56.5)	9 (69.2)	
Neutropenia	Yes	11 (47.8)	3 (23.1)	0.21
	No	6 (26.1)	3 (23.1)	
	Profound	6 (26.1)	7 (53.8)	
Grade of Fever	Low	9 (39.1)	7 (53.8)	0.39
	High	14 (60.9)	6 (46.2)	
ICU Admission	Yes	11 (47.8)	9 (69.2)	0.21
	No	12 (52.2)	4 (30.8)	



A Negative OD <0.120 | **B** Weak positive OD > 0.120 and < 0.240 | **C** Strong positive OD > 0.240

Figure 1: Quantitative detection of biofilm production by MTP – high, moderate and non slime producers differentiated by crystal violet staining in 96 well microtiter plates.



(A) **(B)**

Figure 2: PCR detection of *icaA* (A) and *icaD* (B) genes. Lane 1, molecular size marker (100 bp Ladder); lanes 3 ,4 and 6, *icaA* at 188 bp and *icaD* at 198 bp; lane 8, negative control (DNA template absent).

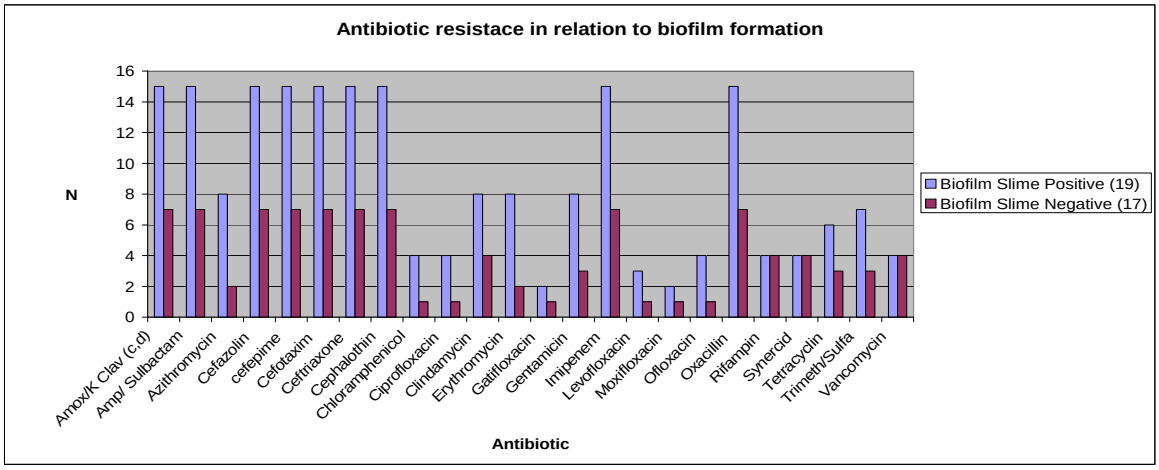


Figure 3: Antibiotic resistance pattern among biofilm producers on CRA in comparison with non biofilm producers. Biofilm forming strains are much more resistant to antibiotics (almost double the resistance pattern presented by non-biofilm forming Staphylococcal strains).

4. Discussion:

Blood stream infection (BSI) remains the major cause of morbidity and death in patients undergoing treatment for cancer; approximately 10% to 30% of all febrile neutropenic cancer patients are bacteremic at presentation (Pizzo *et al.*, 1986 and Hsin *et al.*, 2003) and with the increased use of indwelling venous access devices, catheter-associated bacteremic episodes have become more frequent (Raad and Bodey, 1992). Staphylococci are recognized as the most frequent causes of biofilm-associated infections (Vuong and Otto 2002). In this study bacteremia represented 54.7%, this percent is higher than that stated in literature which range between 10% to 30% (Alexander *et al.*, 2005). According to the global reports, the prevalence of bacteremia in patients with cancer ranged between 5.7-44%. (Kim *et al.*, 2005 and Hosseini *et al.*, 2006). El-Mahallawy *et al.* (2006) performed a prospective cohort study on pediarric cancer patients at National Cancer Institute (NCI) of Cairo that revealed a 46% positive blood culture for bacteremia. The higher

figure in this study may be due to strict inclusion criteria of the patients where we excluded patients who have received empirical antibiotics therapy.

Bacterial strains isolated from blood cultures from febrile pediatric cancer patients had the following distribution: 53 % were Gram negative organisms, 47% were Gram positive; this shows a relatively high percent of Gram negative organisms. Other studies reported that Gram positive organisms accounts for 60 to 70% of bacteremias (Locus *et al.*, 1996; Hughes *et al.*, 1997; Marie *et al.*, 1998 and Hsin *et al.*, 2003). This high percent of Gram positive baceraemia presented by these workers is referred to the factors that possibly contribute to the shift in Gram-positive isolates as increased use of indwelling central venous catheters, fluoroquinolone prophylaxis, and high-dose chemotherapy inducing oral mucositis (*Paganini et al.*, 2003 and Walsh *et al.*, 2006).

In this study the relatively high percent of Gram negative bacteremia; may be explained by the use of more intensive regimens of chemotherapy and the nature of the chemotherapy used has also been reported to influence the bacterial etiology of febrile neutropenia; the use of more specific agents with less cytotoxic potential and, therefore, less mucosal toxicity can lead to a reduction in infections due to Gram-negative organisms. The use of quinolones prophylactic antibiotics in adult cancer patients in Barcelona has shown a sudden resurgence of *E-coli* bacteremia in febrile neutropenic patients. One overlooked factor may even be the regional climactic or environmental conditions that may affect the etiology (Zinner 1999 ; Ramphal 2004).

El Mehallawy and coworkers (2006) explained the high percent of Gram negative organisms forming BSIs in pediatric neutropenic patients at the NCI of Cairo is more likely to be derived from endogenous sources such as gastrointestinal tract and since the high frequency of diarrhea; also the high rate of Gram negative organism due to the nosocomial infection pattern in the institute.

In this Study the Gram negative organisms were distributed as follows: 14% *Klebsiella*, 11% *Proteus*, 9% *E- coli*, 5 % *Enterobacter*, 5% *Yersenia pseudotuberculosis*, 5% *Shigella*, and 3% *Salmonella.* El-Mahallawy and co-workers (2006) stated a similar pattern of microorganisms causing bacteremia in pediatric oncology at NCI of Cairo with predominant *Klebsiella* Species on top of Gram negative isolates. Similar pattern was presented by Ashour and El-Sharif (2009) in a study performed on cancer patients in NCI, Cairo showing the main isolated Gram-negative bacteria from all clinical specimens were *Klebsiella spp.* (31.2%) followed by *Escherichia coli* (22.2%). Also isolation of other less-frequent Gram-negative bacteria had been reported showing the low prevalence of *Salmonella*, *Shigella*, and *Yersenia* species.

El-Mahalawy *et al.* (2006) stated that it's important to recognize the importance of bacteremia due to organisms such as *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella* spp., as they causes higher mortality rate rather than bacteremias due to Gram positive organisms. They reported that 47% of deaths were associated with GNB in contrast to 7% of Gram positive bacteremia and that *Klebsiella* was the more dangerous group with higher mortalities in 88% of hematological malignancies cases.

In the current study the Gram positive cocci were distributed as follows: 18% were *Staphylococcus aureus*, 12 % *S. epidermidis*, 7% *S. hominis*, 4% *S. simulans*, 3% *S. haemolyticus*, 3% *S. warneri*, 1% *S. xylosus*. This pattern was consistent with studies of Ramphal (2004); Kim *et al.* (2005) and Eslaminezhad *et al.* (2010). CONS were the predominant etiological pathogens of bacteraemia. Similar pattern was reported by Ashour and El-Sharif (2007).

The resistance pattern of Gram negative bacteria showed the highest resistance pattern was found with Aztereonam (78%) followed by Cefuroxime (73%) while the highest efficient antibiotics for treatment of GNB were Amikacin with resistance pattern of (13%) followed by Imipenem with (18%). This high pattern of resistance in *Klebsiella* and *E coli* species was reported by Eslaminezhad *et al.* (2010) who stated that isolates of *E. coli and Klebsiella* were multidrug resistant. Similar high resistance pattern was presented by Berjan *et al.* (2001) who found *Enterobacter/Citrobacter/Serratia* group had a 40% - 50% resistance to ceftazidime and piperacillin and 50% of *E. coli* and *Klebsiella* species were resistant to piperacillin with very little quinolone resistance.

Paul *et al.* (2007) also reported similar resistance pattern of Gram-negative bacteria to broad-spectrum beta-lactams, commonly used for empiric treatment of febrile neutropenia, increased with the length of time in hospital prior to bacteremia acquisition. Resistance to ceftazidime increased from 8% when acquired before hospitalization to 48% when acquired after 14 days in hospital.

Generally multi-drug resistance (resistance to three or more antibiotics) was observed in 40% of *S. aureus* isolates than in CONS strains (33%). Antibiotic resistance was (61%) and was related to this group of antibiotics (amoxacillin/K clavulanic, ampicillin/sulbactam, cefazolin, cefepime, cefotaxime, ceftriaxone, cephalothin, imipenem, oxacillin). This was consistent with Kim *et al.* (2005)

who isolated staphylococci blood isolates in pediatrics cancer patients and reported 84% of the isolates were resistant to penicillin and 60% were resistant to oxacillin. The lowest resistance pattern was (8%) reported with gatifloxacin and moxifloxacin and this may be explained by the restricted use of quinolones in pediatrics. Vancomycin resistant staphylococci (VRSA) was recorded in 8 strains (22%) which were 6 strains on *S. aureus* and 2 of *S. epidermidis*. This is consistent with Ashour and El-Sherif (2007) who studied the microbial spectum amd antibiotic susceptibility profile of Gram positive aerobic bacteria from cancer patients at the NCI, in Cairo revealing that 15.5% of *S. aureus* and 11% of CONS resistant to vancomycin. They attributes that the misuse of antibiotics in Egypt might have contributed to this rapid evolution of VRSA strains.

Bacterial biofilm has long been considered as a virulence factor contributing to infection associated with various medical devices and causing nosocomial infection (Aricola *et al*., 2001). Suggested mechanisms by which biofilm producing bacteria cause disease are detachment of cells from medical device biofilm causing blood stream or urinary infection, endotoxin production, resistance to immune system and generation of resistance through plasmid exchange (Donlan and Costerton, 2002).

In the present study we have assayed isolated staphylococcal strains blood isolates for qualitative biofilm forming ability by congo red agar test (CRA). There was 57% strains of *S. aureus* and 50% of CONS biofilm forming. Similar results were reported by Arciola *et. al* (2001) who found that biofilm formation among staphylococci isolated from catheter associated infection was 61 % of *S. aureus* and 49% for *S. epidermidis* isolates, and El-Mahalawy *et al.* (2009) who assayed the staphylococcal blood isolates of febrile neutropenic pediatric cancer at the NCI, Cairo presented 60% of *S. aureus* and 24.4% of CONS were CRA positive. However De Silva *et al.* (2002) reported that only 25% of the tested CONS were biofilm positive by CRA. The discrepancy may be explained by differences in locality and environmental conditions.

In the current study we have performed quantitative detection of biofilm using MTP showed that 57% of *S. aureus* strains were biofilm forming while 41 % strains of CONS were biofilm producers. de Silva *et al*. (2002) reported that 42% of *S. epidermidis* isolated from bacteraemia in neonatal intensive care unit to be positive biofilm producer using MTP. The most important advantage of the microtiter plate assay in addition to the phenotypic biofilm production information presented by CRA is the ability of this method to differentiate between weak and strong biofilm producers. This reflects the severity of the condition and so may help in the determination of suitable line of management, and also at the research level it reflects the degree of gene regulation, as the difference of the degree of biofilm production is due to the difference of PIA production and this is due to changes that occur in the regulation of *ica* operon (Handke *et al.*, 2004).

The molecular mechanism and the genetic control of the PIA synthesis in *Staphylococcal Spp.* have been identified, *icaA, icaD, icaB, icaC*, also termed intracellular adhesion operon (Götz 2002 and Gerke *et al.*, 1998), *icaR* is an additional regulatory gene presented in the same operon. However it have been definitively proven that co-transcription of at least both the *icaA, icaD* genes is required for the N-acetyl-glucosaminyl-transferase activity leading to synthesis of oligomers of no more than 20 residues. (Götz, 2002). In this study 70% of *S. aureus* strains, and 53% CONS, were *icaA* gene and *icaD* gene positive. These results were consistent with Arciola *et al.* (2001) who have shown 61 % of *S. aureus and* 49% for *S epidermidis* were positive and El-Mahallawy *et al.* (2009) also demonstrated that 50% of *S. aureus* and 18% of CONS were simultaneously *icaA* and *icaD* genes positive.

Both the *icaA* and *icaD* genes were present in all biofilm producing strains; this indicates that the presence of both genes is essential for biofilm production and confirms that both genes are part of one operon, so either the entire operon is present or abcsent. This is supported by the results of a study done by Fluckiger *et al.* (2005) who stated that the *ica* locus and biofilm formation are crucial parameters for staphylococcal colonization and survival on implants. Arciola *et al*. (2001) and El-Mahallawy *et al.* (2009) revealed that all strains bearing the *icaA* gene, a component of the *ica* locus, also bear *icaD*.

None of the commensal staphylococcal strains of the control group in this study show the ability to produce biofilm phenotypically and genotypically. These results were similar to Aricola *et al*. (2001) and de Silva *et al.* (2002). This may be explained by the fact that biofilm production is a virulence factor and that the production of PIA is an important component in the process of biofilm formation and the presence of *ica* operon plays an important rule in disease pathogenesis. Biofilm formation in *Staphylococci* is multifunctional and this ability makes strains much better able to survive in hostile environments of tissues and blood. However Eftekhar and Mirmohamadi (2010) found that 8% of the skin isolates of *S. epidermidis* obtained from health volunteers were positive for biofilm production and *icaADBC*. They conclude that *S.*

epidermidis isolates from patients with symptomatic infections are not necessarily more virulent (pathogenic) than the skin contaminants and the capacity to form biofilms in *vivo* is influenced by environmental stimuli, expression levels of *icaADBC* or other regulatory factors independent of PIA synthesis.

One isolates of *S. epidermidis* was positive biofilm producer using MTP but with negative *ica* genes by PCR. Arciola *et al.* (2001) reported this phenomenon and studies performed by Fitzpatrick *et al.* (2005); Rohde *et al.* (2005, 2007); and Kogan *et al.* (2006) highlighted the existence of PIA/PNAG-independent biofilm mechanisms in both *S. aureus* and *S. epidermidis*. as *icaADBC*-independent biofilm mechanism. In the same time four strains (two *S. aureus* and two CONS) were positive *ica* Locus but negative in biofilm slime CRA test. Mack et al. (2000) pointed out that although *ica* genes are responsible for the synthesis of the polysaccharide component, full phenotypic expression of PIA and of biofilm functions could be conditioned by a few additional genes having a direct or indirect regulatory influence. *atlE*, *sarA*, *agrA* and *mecA* are all genes that have been hypothesized potentially to modulate or affect PIA functionality. The appearance of phase-variant bacteria with a complete set of *ica* genes but a slime-negative phenotype, even if relatively infrequent, has been evidenced in several studies during culture on CRA. Finally, it has to be considered that the control of slime production can involve genetic mechanisms capable to alter *ica*-expression acting at a gene level, as for instance, the insertion and precise excision of the naturally occurring insertion sequence IS256 (Ziebuhr *et al.*, 2000).

Comparison of biofilm formation by the two phenotypic methods CRA test and MTP and *ica* gene carriage showed that there were was better agreement between the presence of the *ica* operon and CRA (94.7%) compared to the results obtained for *ica* gene carriage in relation to MTP method (89.5%). These results agree with Aricola *et al.* (2005). El-Mahallawy *et al.* (2009) found a strong agreement between *ica* gene positivity and the ability to produce slime by CRA test ($P < 0.001$). The CRA test is easier to perform with lesser cost.

In the present study, isolates expressing *ica* genes were exhibiting more resistant to broad spectrum antibiotics. This supports that biofilm adds to the virulence profile of *Staphylococcal* strains isolated from blood stream infection. The results were consistent with (De silva *et al.*, 2002, Fux *et al.*, 2004, El-Mahallawy *et al.*, 2009 and Bose et al., 2009). The latter group studied biofilm formation and antibiotic susceptibility on *staphylococci* isolated from different clinical materials and found that there was a significant and clinically relevant higher resistance to conventional antibiotics in biofilm producers than non-biofilm forming. In this study we have found 8 strains (6 strains on *S. aureus* and 2 of *S. epidermidis*) were resistant to vancomycin and this result was supported by the result reported by Bose et al. (2009) where they found that the two strains of *Staphylococci aureus* were vancomycin intermediate resistant *S. aureus* (VISA) and also were biofilm producers. Souli *et al.* (1998) explained that this resistance to vancomycin by biofilm producers may be due to entrapment vancomycin in the extra cellular mucopolysaccharides because of their high molecular weight.

Gilbert *et al*. (2002), reported that biofilm producers were to be 10-1000 times less susceptible towards antibiotics than are the equivalent cells growing planktonically. Also Keren et al. (2004) explained this issue as bacterial populations produces persister cells that neither grow nor die in the presence of antibiotics and that persisters are largely responsible for high levels of biofilm tolerance to antimicrobials. Biofilm hampered penetration of antimicrobial and the concentrations required to eradicate biofilm producing bacteria are higher than those required to eradicate strains that did not produce biofilm (Curtin *et al.*, 2003).

There is association between biofilm production with persistent infection and antibiotic failure. Hence in infection caused by biofilm producing staphylococci, the differentiation with respect to biofilm phenotype might help to modify antibiotic therapy and to prevent infection related to biomedical devices. A suitable and reproducible method is necessary for screening of biofilm in healthcare setting and CRA test is recommended as it is easier to perform, cheap. CRA is a method that could be used to determine whether an isolate has the potential for biofilm production or not. The better agreement between the CRA plate test with the molecular detection of *ica* genes indicates the former as a reliable test for the phenotypic characterization of virulence of clinical isolates.

The continuous evolution of antimicrobial resistanece patterns is bacteria necessiates a comprehensive policy for infection control in hospitals in order to decrease the risk of nosocomial infection in cancer patients.

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Prevalence of SEN Virus Infection in Multitransfused Patients in Assiut University Hospitals, Egypt

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Abstract: SENV is a blood- borne, circular ss DNA virus and possessing nine genotypes (A to I). Among nine genotypes, SENV-D and SENV-H genotypes have the strong link with patients with non (A-E) hepatitis infections. Recently, the identification of SEN virus (SENV) as a possible etiologic agent of parenteral transmission hepatitis let to the study of the prevalence of such agent. This study compared SENV prevalence and its two important genotypes (D&H) which might be pathogenic in high risk subjects including blood transfused patients and hemodialysed patients and low risk subjects as healthy blood donors.

Subjects and methods: This study included 75 multitransfused patients, 60 of them were hemodialysed and the remaining were blood diseased including haemophilics, anaemics and leukemics. The study included also 25 healthy blood donors as a control. They were enrolled consecutively at the department of Internal Medicine, Assiut University Hospital. The sera were separated and SENV DNA was detected by polymerase chain reaction.

Results: A higher prevalence of SENV infection was detected *in* patients groups than in blood donors (46.7% versus 20%).No significant relation was found between SENV infection and age, duration of haemodialysis or liver enzymes. However, there was significant difference between SENV positive and negative patients as regards gender and number of blood transfusions.

Conclusions: SENV is commonly present in blood transfused and haemodialysed patients attended to Assiut University Hospitals as well as in blood donors at comparable rates. SENV infection has been found in only 20% of blood donors but in 46.7% of patients. The results also indicated that other possible routes of SENV infection other than blood transfusion may be included. Its pathogenic role in causing hepatitis is not documented, so far it can be considered as simple guest till further studies have been done.

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Keywords: SEN virus, blood transfusion, haemodialysis, genotyping, prevalence.

1. Introduction:

Hepatitis viruses research started more than fifty years ago (Arie, 1996). As candidates for unknown hepatitis viruses, 2 novel isolates were identified from patients with non (A-E) hepatitis and were designated hepatitis G virus (HGV) and TT virus (TTV) (Nishizawa et al., 1997). When these viruses were discovered, they were expected to account for most of the residual cases of acute or chronic hepatitis that were unrelated to hepatitis (A–E) viruses. Although both HGV and TTV spread universally, there has been no confirmed demonstration of an etiologic association between these viruses and human diseases (Simmonds *et al*., 1998). SEN virus (SENV) is the latest viral agent that has been proposed as a cause of non (A-E) hepatitis. SEN virus is a blood-borne virus that was discovered in 1999 by investigators at DiaSorin Biomolecular Research Institute, Saluggia, Italy, in their search for a viral cause of those cases of post-transfusion hepatitis that are not due to the hepatitis B virus (HBV) or hepatitis C virus (HCV) (Alunan , 1999).

The discovery of SENV by an Italian research group, led by Dr. Daniele Primi, was announced at a press conference in July 1999 without the support of any published empirical data (Allain, 2000). The nomenclature of the virus is derived from the initials of the infected patient, a human immunodeficiency virus–infected injection drug user from whom the virus was first isolated. The first publication of an European patent of the nucleic acid sequence of SENV was on 18 May, 2000 (Primi et al., 2000). Reports of subsequent studies by other investigators were in 2001 (Tanaka *et al*., 2001).

The virus is subgrouped into eight genotypes, SENV- A to H. The ninth genotype (SENV-I) has been identified (Fiordalisi *et al*., 2000). The routes of SENV infection might be mostly parenteral, e.g. transmission by blood transfusion, intravenous drug use or haemodialysis (Umemura *et*

al., 2001a). Transplantation of organs or hematological progenitor cells can also represent a potential risk of infection transmission. SENV has the same transmission modes as HBV and HCV (Yoshida *et al*., 2001).Transfusion significantly increases the relevance of SENV infection. Many studies in different countries revealed that SENV infection is high in patients on maintenance haemodialysis. It is possible; however, that SENV may be transmitted via other means (Hsu *et al*., 2007).

The aim of this study was to determine the prevalence of SEN virus infection among multitransfused patients compared to that of healthy blood donors volunteers in Assiut University Hospitals using polymerase chain reaction (PCR), to determine the genotype of SEN virus detected strains whether SENV-D or SENV-H and also to determine the effect of SEN virus on liver enzymes in multitransfused patients to detect its possible role in causing hepatitis.

2. Subjects and Methods

This study included 75 multitransfused patients, 60 of them were haemodialysed and the remaining 15 were blood diseased including haemophilia, anemia and leukaemia. The study included also 25 healthy blood donors as a control. They were enrolled consecutively at Department of Internal Medicine, Assiut University Hospital and were subjected to clinical examination.

The seventy five patients included in this study were classified into 2 groups, while the third group was the control group: Group I: included 15 blood diseased patients with haemophilia, anemia and leukemia. Group II: included 60 haemodialysed patients. Group III: represented the control group consisting of 25 healthy blood donors. Exclusion criteria: all enrolled subjects were negative for known serologic markers of hepatitis B and C, including IgM antibody to hepatitis B core antigen (anti-HBc), hepatitis B surface antigen (HBsAg) and antibodies to HCV (anti-HCV).

Specimen collection and processing:

Blood specimens (3-5 ml volume) were collected in a clean test tube without any anti-coagulant. Each blood specimen was spun down, within 1 hour of its collection, at 3000 r.p.m. for 10 minutes. Each separated serum was collected and stored at -20ºC.

Extraction of viral DNA:

Viral DNA was extracted from 200 µl serum with QIAamp DNA blood mini kit (Qiagen, Cat. No. 51104- Germany).

Detection of SENV DNA:

SENV DNA was detected by polymerase chain reaction (PCR) with SENV specific primers, as described by Umemura *et al*. (2001a) and Kojima *et al*. (2003). Specific primers **:**(Metabion International AG, D-82152 Martinsried/Deutschland). Sense primer: AI-1F (5'-TWCYCMAACGACCAGCTAGACCT-3'), and antisense primer: AI-1R (5'-GTTTGTGGTGAGCAGAACGGA-3')NB: W= (A or T**),** Y=(C or T), M= (A or C) were used. PCR mixture of 25 µl consisted of: PCR master mix (12.5 µl), forward primer (AI – 1F) (0.5 µl), reverse primer (AI – 1R) (0.5 µl), distilled water (3 µl), extracted DNA (8.5 µl). Amplification was performed for 40 cycles, each included denaturation at 94ºC for 30 sec, annealing at 52ºC for 30 sec and extension at 72ºC for 60 sec. Then 10 min final extension at 72ºC was used to complete strand synthesis.

Genotyping of SENV using PCR:

SENV-D DNA and SENV-H DNA were detected by PCR with SENV specific primers, as described by Kojima *et al*., (2003). Specific primers **:**(Metabion International AG, D-82152 Martinsried/Deutschland). For SENV-D detection, the primers D10S and L2AS were used. Sense primer: D10S (5'-GTAACTTTGCGGTCAACTGCC-3') and antisense primer: L2AS (5'-CCTCGGTTKSAAAKGTYTGATAGT-3') For SENV-H detection, the primers C5S and L2AS were used. Sense primer: C5S (5'-GGTGCCCCTWGTYAGTTGGCGGTT-3') and antisense primer: L2AS (5'-CCTCGGTTKSAAAKGTYTGATAGT-3')

PCR mixture of 25 µl consisted of:

PCR master mix (12.5 µl**),** forward primer (D10S) for SENV-D or (C5S) for SENV-H (0.5 µl**),** reverse primer (L2AS) (0.5 µl**),** distilled water (3 µl), extracted DNA (8.5 µl**).**Amplification was performed for 40 cycles, each included denaturation at 94ºC for 30 sec, annealing for SENV-D at 58ºC for 30 sec, for SENV-H at 50ºC for 30 sec and extension at 72ºC for 60 sec. Then 10 min final extension at 72ºC was used to complete strand synthesis. PCR was performed in a DNA thermal cycler **(**Omnigene TR3, CM220, United Kingdom). For visualization, all of the PCR products generated from PCR amplification were electrophoresed and sized on1.5% agarose gel using 100 bp DNA ladder as DNA molecular weight marker.

Liver function tests:

For measuring alanine aminotransferase (ALT): (BioMed Diagnostics) (Henry, 1964), aspartate aminotransferase (AST): (BioMed Diagnostics) (Tietz, 1976) and total bilirubin (Diamond Diagnostics 30175 Hannover, Germany) (Kaplan *et al*., 1984).

Statistical Analysis:

The data were entered and analyzed using the Statistical Package for Social Science (SPSS) version 16 for windows. For qualitative variables, frequencies and percentages were used.X^2 -test (chi-square test) was used to compare the proportions between the groups. For quantitative variables, mean (x') and standard deviation (SD) were used. Independent T-test, ANOVA and fisher exact test were used to compare between the groups. Significance was discriminated by P value < 0.05.

3. Results

The 75 patients (50 males and 25 females with mean age 45± 13.6) included in this study were classified into 2 groups; one group included 15 blood diseased patients (BD) with leukemia, hemophilia and anemia (3 males and 12 females with mean age 45.8± 18.2). The other group included 60 haemodialysed patients (HD) (47 males and 13 females with mean age 45.4± 12.8). The study was conducted also on 25 healthy blood donors (20 males and 5 females with mean age 31.3± 10.3) as a control group.

SENV was detected in 46.7%of the patients (35 of 75 patients) while it was detected in 20% of the blood donors (5 of 25) with a statistically significant difference as shown in table (1). SENV was detected in 45%of haemodialysed patients (27 of 60 patients) while it was detected in 53.3% of blood diseased patients (8 of 15) as shown in table (2). SENV DNA positive samples showed one band at 349 bp DNA fragment when examined by gel electrophoresis (Fig. 1A).

SENV-D was detected in 14.3%of SENV positive patients (5 of 35 patients ; 3 haemodialysed patients and 2 blood transfused patients) while it was detected in all of SENV positive blood donors (100%) as shown in tables (3&4). SENV-D DNA positive samples showed one band at 231 bp DNA fragment when examined by gel electrophoresis (Fig. 1B).

SENV-H was detected in 14.3%of SENV positive patients (5 of 35 patients; 3 haemodialysed patients and 2 blood transfused patients). Whereas none was detected in the control group as shown in tables (3&4). SENV-H DNA positive samples showed one band at 230 bp DNA fragment when examined by gel electrophoresis (Fig. 1C).

SENV-D/H co-infection was detected in 57.1%of SENV positive patients (20 of 35 patients; 19 haemodialysed patients and 1 blood transfused patient), whereas none was detected in the control group as shown in tables (3&4).

There is no significant difference in mean age between SENV positive and negative patients while it is evident significant difference between SENV positive patients and SENV negative patients, as regards gender (Table 5).

Concerning the risk factors for patients, it was found highly significant statistical difference regarding the mean number of blood transfusions which was 2.33± 0.6 vs. 11± 7.6 in SENV positive and negative patients respectively (Table 5). The mean number of blood transfusion was nearly similar among SENV – D positive patients, SENV –H and SENV –D/H patients (2.9, 2.5 and 2), respectively, as shown in table (6).

The mean duration of haemodialysis was 8.1± 4.9 in SENV positive patients vs. 7.2± 2.7 in SENV negative patients with no significant statistical difference between them (Table 5). Higher mean concerning duration of haemodialysis was noticed among SENV–D positive patients than among SENV- H or SENV- D/H patients (10± 2.1 vs. 6± 1.4 and 8.1± 4.9) respectively as shown in table (6).

The biochemical parameters as regards liver enzymes (ALT, AST and total bilirubin) didn't significantly differ between SENV positive and negative patients as shown in table (5). Table (6) showed no statistically significant difference between SENV – D positive, SENV – H positive and SENV – D/H positive patient as regards AST, total bilurbin and mean duration of haemodialysis. However, mean AST of SENV – H infected patients was slightly higher than other types of infection .However, statistically significant difference occurred among SENV- D positive , SENV –H positive and SENV – D/H positive patient as regards ALT in spite of their normal values.

Concerning the two patients groups and the liver enzymes values, there is no statistically significant difference either between SENV positive and negative haemodialysed patients or blood transfused patients (Table 7). Table (8) showed no statistically significant difference between SENV positive and negative blood donors.

Table (1): Prevalence of SENV infection using PCR in patients and control groups

SENV infection	Patients (n=75)	Control (n=25)	*P*-value*
Positive	35(46.7%)	5(20%)	0.02
Negative	40(53.3%)	20(80%)	

Fisher exact test*

Table (2): Prevalence of SENV infection in different patients groups and the control group

Group	No. of subjects	SENV positive	SENV negative	*P*-value*
Haemodialysed patients	60	27(45%)	33(55%)	0.56
Blood diseased patients	15	8(53.3%)	7(46.7%)	0.63
Control group	25	5(20%)	20(80%)	0.0001

Fisher exact test*

Table (3): Prevalence of SENV genotypes in SENV positive subjects groups.

	SEN V positive		*P*-value*
	Cases (n=35)	Control (n=5)	
SENV-D	5(14.3%)	5(100%)	0.0006
SENV-H	5(14.3%)	0(0%)	
SENV-HD	20(57.1%)	0(0%)	
Negative for SENV- D or H	5(14.3%)	0(0%)	0.35

Fisher exact test*

Table (4): Prevalence of SENV genotypes in patients and control groups.

Group	SENV-D	SENV-H	SENV-D/H	Negative for SENV- D/H
Haemodialysis patients	3 (11.1%)	3 (11.1%)	19 (70.4%)	2 (7.4%)
Blood diseased patients	2 (25%)	2 (25%)	1 (12.5%)	3 (37.5%)
Control group	5 (100%)	0 (0%)	0 (0%)	0 (0%)

Table (5): Relationship between gender,age, risk factors, liver enzymes and SENV infection

The variable	SENV Positive (N=35)	SENV Negative (N=40)	*P*-value
Gender • **Male** • **Female**	 18 (51.4%) 17 (48.6%)	 32 (80%) 8 (20%)	0.003*
Age	50.6 ± 10.1	44.1 ± 9.6	0.09*
Risk Factors • **Duration of haemodialysis (years)** • **No of blood transfusions (units)**	 8.1 ± 4.9 2.33 ± 0.6	 7.2 ± 2.7 11 ± 7.6	 0.4 0.0001*
Liver function tests • **ALT (U/L)** • **AST (U/L)** • **Total Bilirubin (mg/dl)**	 7.4 ± 5.2 11.9 ± 4.1 0.26 ± 0.03	 8.7 ± 4.9 9.1 ± 4.5 0.22 ± 0.02	 0.5 0.1 0.7

AST, aspartyl transaminase, ALT, alanine amino-transaminase.
*Fisher exact test

Table(6):Relationship between SENV genotypes infection and different variables in the patient groups.

The variable	D (n=5)	H (n=5)	H\D (n=20)	P-value
Risk Factors • **Duration of haemodialysis** • **Amount of blood transfusion**	10 ± 2.1 2.9	6 ± 1.4 2.5	8.1 ± 4.9 2	0.08*
Liver Function Tests • **ALT (U/L)** • **AST (U/L)** • **Total Bilirubin (mg/dl)**	4 ± 1.3 10 ±4.2 0.02 ± 0.008	10.5 ± 9.2 17.5 ± 2.1 0.02 ± 0.0001	7.1± 5.02 11.5 ± 3.8 0.3 ± 0.03	0.04** 0.1** 0.7**

AST, aspartyl transaminase, ALT, alanine amino-transaminase. *Fisher exact test, ** ANOVA

Table (7): Multitransfusion and haemodialysis impacts on the prevalence of SENV infection and the liver function tests in patients groups.

	Liver Function test	SENV Positive	SENV negative	p-value
Haemodialysed patients	ALT (U/L) AST (U/L) Total Bilurbin (mg/dl)	7.5 ± 5.4 12.2 ± 4.3 0.2 ± 0.03	9.8 ± 5.4 9.6 ± 4.9 0.2 ± 0.1	0.5 0.06 0.8
Multi-transfused patients	ALT (U/L) AST (U/L) Total Bilirubin (mg/dl)	7.3 ± 5.8 11 ± 3.5 0.5 ± 0.3	5.7 ± 3.3 7.9 ± 3.2 0.3 ± 0.08	0.6 0.4 0.7

AST, aspartyl transaminase, ALT, alanine amino-transaminase

Table (8): Comparison of SENV positive and negative blood donors (control subjects) as regards liver enzymes.

Liver Function tests	SENV positive (No.=5)	SENV negative (No.=20)	p-value*
ALT (U/L)	5.5 ± 2.1	5.4 ± 3.1	0.9
AST (U/L)	9 ± 3.4	9.9 ± 2.1	0.9
Total Bilirubin (mg/dl)	0.4 ± 0.3	0.3 ± 0.08	0.7

AST, aspartyl transaminase, ALT, alanine amino-transaminase

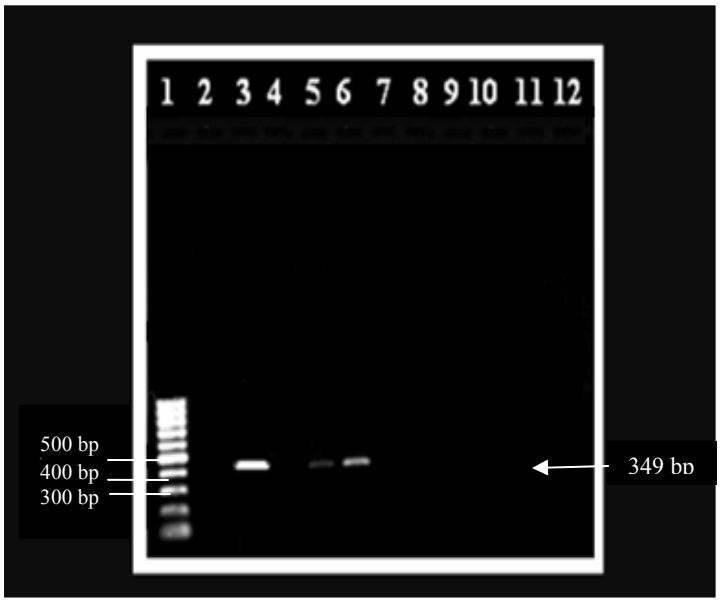

Figure (1A): **Detection of SENV DNA**
Lane (1): DNA 100 bp ladder.
Lane (2): Negative control.
Lane (3): Positive control.
Lanes (5) and (6): Positive cases.
Lanes (4), (7)-(12): Negative cases.

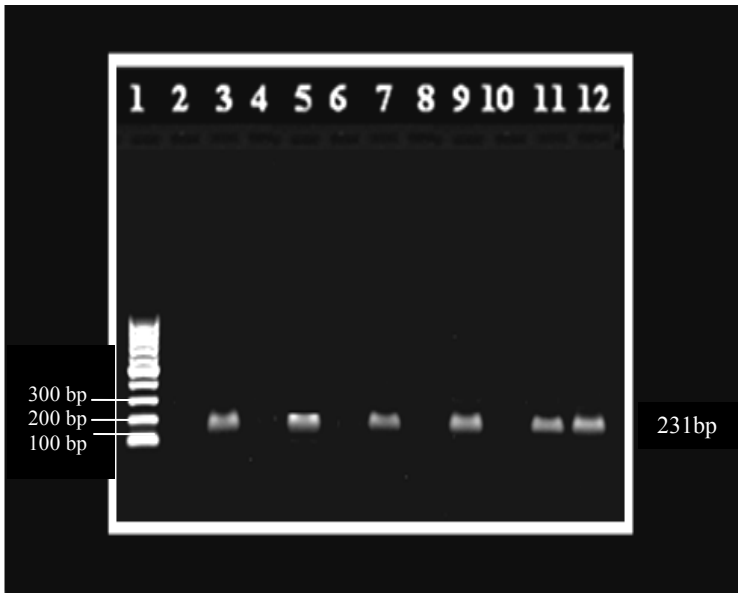

Figure (1B): **Detection of SENV-D DNA**
Lane (1): DNA 100 bp ladder.
Lane (2): Negative control.
Lane (3): Positive control
Lanes (4), (6), (8) and (10): Negative cases.
Lanes (5), (7), (9), (11) and (12): Positive cases

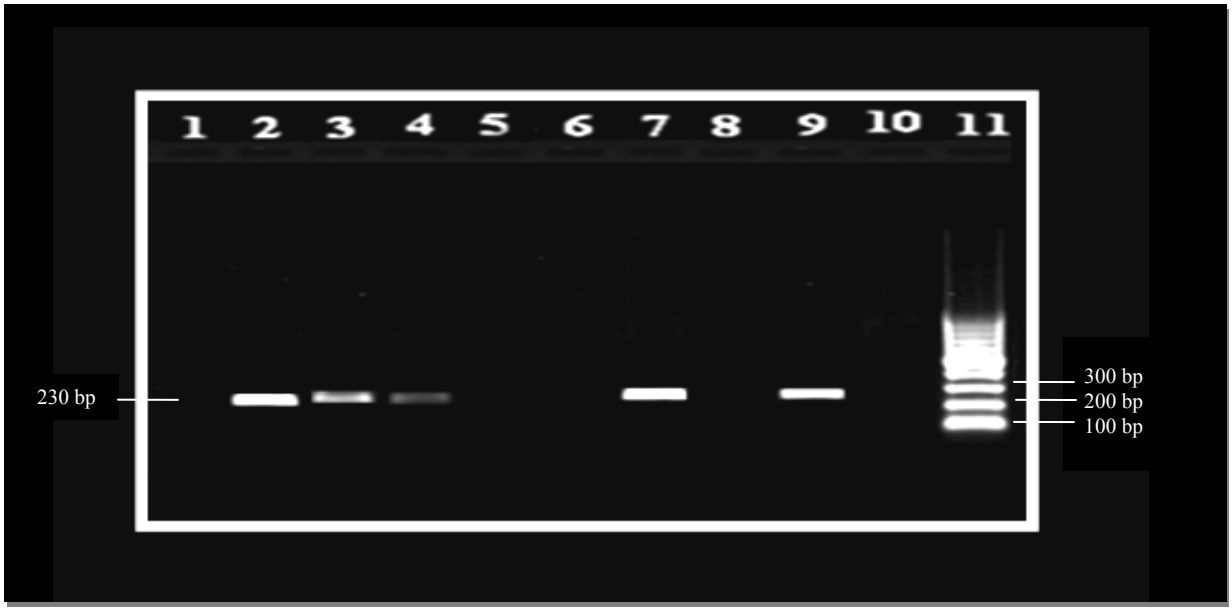

Figure (1C): Detection of SENV-H DNA
Lane (1): Negative control.
Lane (3), (4), (7) and (9): Positive cases.
Lane (11): DNA 100 bp ladder.
.Lane (2): Positive control.
Lanes (5), (6), (8) and (10): Negative cases.

4. Discussion:

SENV is a blood- borne ,circular ss DNA virus and possessing nine genotypes (A to I). Among nine genotypes, SENV-D and SENV-H genotypes have the strong link with patients with non (A-E) hepatitis infections (Karimi-Rastehkenari and Bouzari, 2010).

The current study compared the prevalence of SENV in blood transfused, haemodialysed patients and blood donors. The study was conducted on 75 patients (50 males and 25 females) and 25 healthy blood donors (20 males and 5 females). SENV was detected in 46.7% of patients compared to 20% of the blood donors. Many investigators in different countries mentioned data in agreement with the present study as infection rates in the blood donors were 10-20% in Japan (Shibata et al .,2001), 14-20% in Taiwan (Kao *et al*., 2002), 13% in Italy (Pirovano et al., 2002), 24% in Greece (Umemura *et al*., 2003), 31% in China (Mu *et al*., 2004), 16% in Egypt (Sayed et al., 2006), 25% in Turkey (Serin *et al*.,2006) and (Sharifi *et al*.,2008).

However, this rate was higher than that of blood donors in other reports from different parts of the world; in Italy (2%) (Mushahwar, 2000), in the United States (1.8%) (Umemura *et al*., 2001a), in Germany (8%) (Schröter *et al*., 2002) and (10%) in Egypt (Loutfy *et al*., 2009). The reasons for these demographic differences are unclear, but they make it essential to compare case and control subjects from areas of similar endemicity (Umemura *et al*., 2003).

Patients on maintenance haemodialysis (HD) should be at increased risk of such an infection, as they are for HDV and HBV infections (Wreghitt, 1999) since the virus may be transmitted parenterally (Umemura *et al.,* 2001a). In support of this view, the overall detection of SENV DNA in HD patients of this study was higher than that observed in healthy blood donors (45% vs. 20%).This is in agreement with Kobayashi *et al*., (2003) who reported a prevalence of 37.6% among HD patients in Japan, Dai *et al*., (2005) reported 61.6% in Southern Taiwan, Pirovano et al., (2005) reported 40.9% in Italy and Loutfy *et al*., (2009) reported 52.4% in Egypt. In reverse, other investigators reported lower prevalence as Schröter *et al*., (2003) who reported prevalence of 10.9% among HD patients and Hsu *et al*., (2007) reported prevalence of 27.7% among peritoneal dialysis (PD) patients.

In the present study, the prevalence of SENV among blood transfused patients (patients with hemophilia, anemia and leukemia) was higher than that of blood donors (53.3% vs.20%). This is in agreement with Kao *et al*., (2002) who reported that the prevalence of SENV among blood transfused patients was high being 68% in hemophilic patients and 90% in thalassemic patients. These patients were frequently exposed to blood and blood products, confirming the importance of the parenteral route for SENV transmission. This is in agreement with Karimi-Rastehkenari and Bouzari, (2010) who reported that SENV viremia was significantly higher among thalassemic patients than healthy individuals.

On the other hand, Umemura *et al*., (2003) reported lower prevalence (35%) in patients with hepatitis associated aplastic anemia (HAA).

The present study revealed that SENV-D was the only genotype detected in all SENV positive subjects of the control group (100%) while SENV-H was not detected in this group. Therefore, combined infection was not detected in this group. This is in agreement with a study done in US by Umemura *et al*. (2001b) and also in Japan and Greece (Umemura et al., 2003). In contrast to the present investigation, SENV-H was the predominant strain in blood donors in the US (Umemura *et al*., 2001b) and Taiwan (Kao *et al.,* 2002). Schröter *et al*. (2002) reported that the prevalence of SENV-H was 16.8% among blood donors. Thom et al. (2010) detected SENV –H prevalence in 45.4% of Ghanaian blood donors.

Different rates of the two genotypes (D&H) were mentioned by other investigators. The prevalence of SENV-D mentioned by Kobayashi *et al*, (2003) was 77%, that of SENV-H was 15% and that of both SENV-D/H was 8% in the control group. In Turkey, Serin *et al*., (2005) detected SENV-D in 2 (40%) of 5 SENV positive subjects and SENV-H was detected in 3 (60%) of 5 SENV positive subjects in the control group.In another study, Serin *et al*., (2006) reported that the prevalence of SENV-D was 10% while SENV-H was 15% in the blood donors.

In a study done in Egypt by Sayed *et al.,* (2006), SENV-D was detected in 4 % of blood donors while SENV-H was detected in 12%of blood donors. Co-infection with both variants was not detected in the blood donors, this supported the present study. Borawski *et al*. (2006) detected SENV-H in 2 % of control subjects in Poland. In Iran, Sharifi *et al*. (2008) detected SENV-D/H in 23% of blood donors, while Karimi-Rastehkenari and Bouzari (2010) reported that frequency of SENV-H viremia was significantly higher than SENV-D among healthy individuals.

All these variations may be referred to that high prevalence of SENV infection can be attributed to only some of the SENV strains; for example, SENV-B, SENV-A and SENV-E which are found among healthy blood donors and do not appear to be related to non (A-E) hepatitis (Allain *et al*., 2002).This discrepancy is postulated to be due to geographical distribution of SENV variants and differences between regions in the same country (Dai *et al*., 2005).

Either SENV-D or SENV-H was detected in SENV positive patients in a similar percentage (14.3%). However, combined infection with both variants was higher than either genotype monoinfection (57.1% vs. 14.3%). The results of this study were in agreement with a study done by Quiros Roldan *et al*., (2005) who found SENV-D and SENV-H in a similar percentage (16%). In contrast, , many investigators as Kao *et al*., (2002) reported that the prevalence of SENV-H was 2-7 times higher than that of SENV-D in different subjects, and mixed SENV-D/H infection was not common. Schréter *et al*., (2006) showed 34.7% SENV-H positive cases.

A study on the prevalence of SENV among patients undergoing haemodialysis (HD) in Poland revealed that SENV-H viraemia was prevalent in 40% of HD patients (Borawski *et al*., 2006). The present study revealed that either SENV-D or SENV-H was detected in SENV positive patients on HD in a similar percentage (11.1%). The proportion of SENV-H positive HD patients is similar to that found in HD patients from Germany (12.8%) (Schröter *et al*., 2003), but lower than that found in Japanese HD patients (38%) (Kobayashi *et al*., 2003).

These results strongly suggest that infection with one SENV variant most likely does not protect against infection with another SENV variant (Pirovano *et al*., 2005). In Egypt, as previously reported by Loutfy *et al*., (2009), 61% of HD patients were positive to SENV-H only,4% were positive to SENV-D only, and 36% were positive for both SENV-H and SENV-D. Since patients who have been on HD for the longest period of time are likely to have received more blood transfusion, one could expect that the presence of SENV DNA correlates with the HD treatment.

It was demonstrated from this work that there was no relationship between SENV positivity and the length of time on HD. This indicated that the blood transfusion may not be the only important route of SENV transmission in these patients but also other routes may be included. Evidence to support transmission of SENV by blood transfusion has been reported (Shibata et al., 2001).

In the present investigation, the number of blood transfusions in SENV positive patients was lower than that of SENV negative patients denoting that the number of blood transfusions is probably not a risk factor. Similar result is obtained by Loutfy *et al*., (2009) who revealed no association between SENV infection and duration of hemodialysis. However, Schréter *et al*. (2006) found that the number of blood transfusions was significant risk factor.

The present work revealed that there was no association between age and prevalence of SENV infection as there was no significant difference between SENV positive patients and SENV negative patients as regards age. However, some authors described an age – specific prevalence of SENV in adults (Kao et al., 2002).

SENV infection was found in nearly similar proportions among males and females. In spite of this, significant difference was noticed between SENV positive patients and SENV negative patients as regards gender. This was in agreement with data of many investigators, Yoshida *et al*. (2002) mentioned no significant differences in age and gender between SENV positive and SENV negative patients with non B and non C chronic liver disease. Results of many other studies support the previous data (Mikuni *et al*., 2002; Pirovano *et al*., 2002; Kobayashi *et al*., 2003; Quiros Roldan *et al*., 2005; Serin et al., 2005 and Borawski et al., 2006). In Egypt, Loutfy et al., (2009) revealed no association between SENV infection and age or sex of HD patients. In contrast, Kobayashi *et al*., (2003), Chiou *et al*., (2006), Schréter *et al*., (2006) and Spataro *et al.* (2006) described a notable difference in SENV prevalence according to gender with a higher proportion of males among SENV positive patients.

On comparison the laboratory parameters of liver injury in SENV positive and negative patients, it was not observed any differences in liver *al* enzymes values (ALT, AST and TB). Similar to other studies (Shibata *et al*., 2001; Umemura *et al*., 2001b; Yoshida *et al*., 2002; Kao et al., 2002; Kobayashi *et al*., 2003; Mu *et*., 2004; Sagir *et al.,* 2004; Borawski *et al*., 2006 and Schréter *et al*., 2006), it has not been observed any influence of SENV infection on the worsening of laboratory findings in the patients group, hence no confirmation of a pathogenic role of SENV in liver injury.

Chiou et al. (2006) reported also that SENV viraemia was not associated with elevated liver enzymes in thalassemia patients. High levels of ALT, AST and TB in SENV positive patients compared with SENV negative patients have been reported. However, these findings were not statistically significant (Pirovano *et al*., 2002 and Serin *et al*., 2005). So they concluded that SENV did not seem to contribute to the pathogenesis of liver diseases.

5. Conclusions:

SENV is commonly present in blood transfused and haemodialysed patients attended to Assiut University Hospitals as well as in blood donors at comparable rates. SENV infection has been found in only 20% of blood donors but in 46.7% of patients. The results also indicated that other possible routes of SENV infection other than blood transfusion may be included. Its pathogenic role in causing hepatitis is not documented, so far it can be considered as simple guest till further studies have been done.

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The Effectiveness of Kangaroo Technique on Preterm Baby Weight Gain

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Abstract: The aim of the study was to assess mother's perception about kangaroo technique, implement on hospitalized premature babies and evaluate the effectiveness of kangaroo technique on preterm babies weight gain. A quasi experimental design was used in this study. The study subjects consisted of two hundred (200) mothers divided into two identical groups. The studied group included mothers who applied the kangaroo technique, while those exposed to routine hospital care were consider a control. Data were collected through using pre-designed interviewing questionnaire to assess mothers and neonates characteristics, knowledge about kangaroo technique. An observational checklist was used to assess mothers' practices; towards application of kangaroo technique. This technique had been applied for the study group only. The result of the study revealed that there was a statistically significant difference in mother's knowledge and practices between both study and control groups after application of kangaroo technique with significant effects on preterm baby weight and attachment. The study concluded that application of kangaroo technique enhanced mother-child attachment and had positive effect on weight gain and possibility of early discharge from neonatal intensive care units (NICUs). Therefore, the study recommended the application of kangaroo technique for all low birth weight premature babies as part of the routine daily care to babies admitted to the neonatal intensive care units.

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Keywords: Kangaroo technique- Premature babies-Mother infant bonding-Duration of hospitalization- weight gain.

1. Introduction:

Kangaroo care is a technique practiced on newborn, usually preterm babies wherein the infant is held, skin-to-skin, with an adult. Kangaroo care for preterm babies may be restricted to a few hours per day, but if they are medically stable that time may be extended. Some parents may keep their babies in-arms for many hours per day. Kangaroo care, named for the similarity to how certain marsupials carry their young, was initially developed to care for preterm babies in areas where incubators are either unavailable or unreliable.[1] Kangaroo care seeks to provide restored closeness of the newborn with mother or father by placing the infant in direct skin-to-skin contact with one of them. This ensures physiological and psychological warmth and bonding. The kangaroo position provides ready access to nourishment. The parent's stable body temperature helps to regulate the neonate's temperature more smoothly than an incubator, and allows for readily accessible breastfeeding.[2]

Beginning kangaroo care within the first 2 hours after birth seems to be the most effective time period for successful breastfeeding. Many advocates of natural birth encourage immediate skin-to-skin contact between mother and baby after birth, with minimal disruption. Babies must be kept warm and dry. This method can be used continuously around the clock or for short periods per day gradually increasing as tolerated for babies who are compromised by severe health problems. It can be started at birth or within hours, days, or weeks after birth. Proponents of kangaroo care encourage maintaining skin-to-skin contact method for about six weeks so that both baby and mother are established in breastfeeding and have achieved physiological recovery from the birth process.[3]

Kangaroo care is beneficial for parents because it promotes attachment and bonding, improves parental confidence, and helps to promote increased milk production and breastfeeding success.[2,4,5] Kangaroo care arguably offers the most benefits for preterm and low birth weight babies, who experience more normalized temperature, heart rate, and respiratory rate[6], increased weight gain[7], fewer nocosomial infections and reduced incidence of respiratory tract disease.[5] Additionally, studies suggest that preterm babies who experience kangaroo care have improved cognitive development, decreased stress levels, reduced pain responses, normalized growth, and positive effects on motor development.[8,9,10] Kangaroo care also helps to improve sleep patterns of babies, and may be a good intervention for colic.[11] Earlier discharge from hospital is also a possible outcome.[12] Finally, kangaroo care helps to promote frequent breastfeeding, and can enhance mother-infant bonding.[13]

Kangaroo care often results in reduced hospital stays, reduced need for expensive healthcare technology, increased parental involvement and teaching opportunities, and better use of healthcare dollars. Overall, kangaroo care helps to reduce morbidity and mortality in developing countries, provides opportunities for teaching during postnatal follow-up visits, and decreases hospital-associated costs. [1]

The aim of the study is to:

- Assess mother's perception about kangaroo technique.
- Implement kangaroo technique on hospitalized premature newborns.
- Evaluate the effectiveness of kangaroo technique on mother-baby attachment and preterm baby weight gain.

2. Subjects and Methods:

Subjects and methods of this study are portrayed under four main topics as follows:

Technical design
Operational design
Administrative design
Statistical design

I. Technical Design:

The technical design for the study includes research design, research settings, subjects and tools of data collection.

Research Design

A quasi experimental design was used in this study to help gaining information about the effect of kangaroo care on the neonate and the mother, and to compare control cases with experimental cases.

Research Settings:

The study was conducted in the NICUs at Maternity and Gynecological Hospital affiliated to Kasr El-Aini Teaching Hospital-Cairo University and Maternity and Gynecological Hospital affiliated to Ain Shams University Hospitals.

Subjects:

The study subjects consisted of two hundred (200) mothers and their preterm neonates. The subject was assigned randomly to two groups, the intervention (100 mothers & neonate pairs) and control (100 mothers & neonate pairs). The following were the inclusion criteria:

All premature babies weighing from 1.000 gm to 2.500 gm regardless the type of feeding.

Free from congenital anomalies, heart diseases, surgical problems, neonatal jaundice, hypoxic ischemic insult or large for gestational age.

Not on assisted ventilator support.

Mother which willing to participate in the study.

Tools of Data Collection:

Data were collected using the following tools:

1- An interviewing questionnaire: It was designed by the researcher and written in simple Arabic language. It is composed of the following parts to collect data in relation to:

Part I: Characteristics of the studied mothers including age, qualification, residence area, occupation and the history of previous pregnancies such as history of intrauterine fetal death, history of abortion or history of neonatal death.

Part II: Characteristics of the studied neonate including gender, gestational age, birth weight and ranking.

2- Mother's knowledge regarding to kangaroo technique:

It includes general knowledge about kangaroo, definition of kangaroo, and importance of kangaroo for the mothers and for the baby.

Questions were in the form of multiple choices. The time consumed to fill in the questionnaire by the researcher for each mother in study and control groups was 15-20 minutes.

The total score level for the questionnaire sheet was "100" marks.

- The mother's answer scores were categorized into either:

Score < 60: poor knowledge.
Score from 60 < 75: average knowledge.
Score 75 to100: good knowledge.

3- Observational checklist:

An observation checklist for mother's kangaroo care practices was developed by the researchers to evaluate mother's skill for implementation of kangaroo care. This tool was developed by the researchers in form of steps and was conducted for each mother individually, in a pre-selected warm, calm environment. At the beginning a pre-test was conducted to assess the mother's knowledge and skills about the kangaroo care technique, followed by giving instruction and teaching about the Kangaroo technique, after explaining its purpose, benefits, effects on the neonate-mother bond and attachment; then the kangaroo care was applied for each baby after being sure that he/she is clinically stable. The observation checklist was used for four successive times weekly

for a month to insure accuracy mother's skill evaluation.

4- Monitoring preterm baby weight.

II. Operational Design:

The operational design included preparatory phase, pilot study, and field work.

Preparatory Phase

A review was done of the past and current available relevant literature, to cover the various aspects of the problem, and to design the study tools for data collection.

Pilot Study

A pilot study was conducted on 10 premature neonates and their mothers to evaluate the content of questionnaire. The tool was tested on those premature and their mothers who fulfilled inclusion criteria. As a result of the pilot study there was a need to add control cases. Subjects who shared in the pilot study were excluded from the study main sample.

Field work

Data were collected throughout one year period; data collection of this study was carried out from beginning of May 2005 to beginning of May 2006. The researchers were available daily from 9.00 am to 3.00 pm. Each mother delivering a neonate who was admitted to the NICU and fulfilling the inclusion criteria was interviewed individually and assessed using the previously mentioned study tools. The study groups were exposed to kangaroo care intervention while the control groups were exposed to routine hospital care.

III. Administrative Design:

An official permission was obtained from chairman of the NICU to conduct this study.

Ethical Consideration:

A verbal consent was obtained from mothers included with their babies in the kangaroo technique intervention.

IV. Statistical Design:

Data were revised, coded, tabulated and analyzed using numbers and percentage distribution and carried out in a PC computer.

The following statistical techniques were used:

Percentage.

Mean.

Standard deviation.

T- Test for quantity variables.

Chi-square (X^2) for qualitative variables.

Paired t-test for comparison of paired two quantity variables.

Significance of the Results:

When $p > 0.05$ it is statistically insignificant difference.

When $p < 0.05$ it is statistically significant difference.

When $p < 0.01$ or $p < 0.001$ it is high statistically significant difference.

3. Results:

Table (1) shows that, there was no statistically significant difference between the study and control groups by their total score level regarding to kangaroo care as X2 =2.9 at p- level >0.05.

Table(2) shows that, there was highly statistically significant difference in mother's knowledge pre and post intervention regarding kangaroo care definition, important for both baby and mother as $X^2 = 21.3$ at p level <0.001.

Table(3) shows that, there was a statistically significant difference in weight change of the premature babies of the study subjects at the 2nd, 3rd, & 4th week (t-test = 11.766, 22.996 & 15.291 respectively at p= 0.000).

Table (4) shows that there was a statistically significant difference in weight change of the premature babies of the study subjects at the 2nd, 3rd, & 4th week (t= 11.525, 16.399, & 19.772 respectively at p= 0.000).

Table (5) shows that, there was no statistically significant difference between the study and control groups regarding to their mean weight on admission (often one week), but after the first week, there was highly statistically significant difference in mean score for weight at p level <0.0001.

Table (1): Distribution of both Experimental and Control Groups by their Total Score Level Regarding to Kangaroo Care Technique.

Item	Study		Control		X^2	P value
	No= 100	%	No= 100	%		
Good	7	7	2	2		
Average	23	23	24	24	2.9	>0.05
Poor	70	70	74	74		

Table (2): Distribution of Experimental Groups by their Knowledge Score Level Regarding to Kangaroo Care Technique.

Knowledge Score	pre		post		X^2	P value
	No= 100	%	No= 100	%		
Good	7	7	90	90		
Average	23	23	8	8	21.3	<0.001
Poor	70	70	2	2		

Table (3): Weight Change in the Experimental Group from Week to Week

Study Group I	Weight Change		Paired t-test	
	Range	Mean±SD	t	P-value
After 1st Week	120.00 - 200.00	149.40±13.05		
After 2nd Week	140.00 - 205.00	166.50±13.27	-11.766	0.000
After 3rd Week	160.00 - 210.00	184.60±10.77	-22.996	0.000
After 4th Week	20.00 - 210.00	192.90±26.78	-15.291	0.000

Table (4): Weight Gain for Control Group During the first 4 Weeks (n=100).

Control Group II	weight Change		Paired t-test	
	Range	Mean±SD	t	P-value
After 1st Week	40.00 - 150.00	69.40±23.15		
After 2nd Week	60.00 - 130.00	93.47±21.23	-11.525	0.000
After 3rd Week	70.00 - 140.00	110.10±16.30	-16.399	0.000
After 4th Week	40.00 - 140.00	122.81±15.66	-19.772	0.000

Table (5): Comparison between Experimental and Control Groups by their Weight Gain (n=200).

Weight	Study group	Control group	T-test	
	Mean±SD	Mean±SD	t	P-value
On admission	1436.08±442.31	1436.08±442.31		
After 1st measure	1336.20±416.51	1336.20±416.51		
After 1st Week	1467.20±457.46	1405.60±421.56	0.990	0.323
After 2nd Week	1655.50±416.92	1507.24±425.06	2.478	0.014
After 3rd Week	1853.90±447.51	1625.83±427.77	3.645	0.000
After 4th Week	2023.80±449.11	1748.65±428.77	4.384	0.000

4. Discussion:

The results of applying kangaroo technique on knowledge and attachment, among both studied groups, showed that there were statistically insignificant difference between study and control groups as regards their knowledge related to concept of KC and its importance for the baby. However, the difference in knowledge in the study group pre and

post kangaroo technique showed a highly statistically significant difference meaning that simple education for those mothers was very fruitful and had a positive impact on them.

Almost all the mothers in the study group had no information about kangaroo care techniques as in the pre intervention either they gave wrong answers or they had no answers about definition of KC and its importance for the mother and the baby. Accordingly there was highly statistically significant difference in comparison between pre and post practice assessment.

Analysis of the results of the mother-neonate attachment revealed that, there was highly statistically significant difference in the score obtained in the questionnaire before and after the practicing of KC, this means that the attachment between the premature baby and his/her mother has significantly increased with the application of KC.

In a similar study, Wallace, and Marchall (2001), found that skin to skin contact between mother and newborn babies promotes maternal-neonate attachment. Similarly, Spanjer (2002) stated that KC increases togetherness, that which is far removed of the threat of separation, it provides a sense of containment and closeness, in addition mothers are more quickly adapted to the appearance of their babies, strengthening the mother confidence in gaining control over her emotions, her competencies in mothering skills and her perception of herself as a good mother.

Previously, Ludington-Hoe and Golant (1993) stated that, KC facilitates bonding, enhances warm melting and loving sensation that comes with bonding and the mother will sooner feel affectionate relationship.

Analysis of the results of the current study questionnaire showed that there was highly statistically difference in mothers' response in pre and post test of the study group, while there was no difference in the control group. These results are explained by the fact that this difference before and after the practicing of KC is due to the effect of the procedure that lead to enhancing the attachment and bonding between the mother and the baby and increasing mothers knowledge about the benefits of KC.

The present study results revealed that weight of the premature neonates of the experimental group showed better increase from time of applying the technique, this is a definite proof that the kangaroo mother care was effective and improved the neonate feeding, mothers' milk production and provided easy accessibility to the mother breast.

Despite that the weight of neonates of the control group who did not practice the KC were increasing from week to week due to the normal growth of the neonate and the routine care provided in the NICU, yet there was a statistically significant change in the weight of the premature baby in the study group after the practice of KC compared to the control group. This finding is directly related to the effect of Kangaroo care that is believed to improve the neonate weight through increasing the milk production, and neonate accessibility to the breast, as demonstrated by (Padden, Glen and WHO 1997) which reported that, the kangaroo care leads to successful lactation because of increased hormonal and sensory stimulation of the mothers milk production, that causes increase in the neonate weight, and prevention of hypoglycemia.

5. Conclusion:

Based on the study findings it could be concluded that:

Mother's knowledge and practice towards kangaroo care technique had been improved after the implementation of the technique by the researcher. There was a highly statistically significant difference between the mean weight gain of the study group who received the kangaroo care by their own mothers and the control group who received the routine care of NICUs; with a direct proof to what extent the kangaroo care implementation had a positive effect on weight gain. The researcher detected to what extent the technique had increased the mothers and their neonatal attachment.

Recommendations

The study recommends that:

Kangaroo care technique should be part of the routine care of all premature and, low birth weight babies admitted to NICUs.

An illustrated leaflet demonstrating step by step kangaroo care technique should be distributed to all neonatal intensive care units all over the country to be followed by neonatal intensive care staff and should be adopted as a hospital protocol for neonatal care.

Training courses for health care providers on the importance and benefits of kangaroo care technique should be implemented on a wide scale.

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The Contribution of Women in Rural Development in Iran

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Abstract: This paper highlights the concerns of women and the challenges they face in rural development process. Agriculture is certainly a major contributor to rural development in many countries. It is one of the most important economic sectors in Iran. In this way rural women play a special role in rural development. When women are economically and socially empowered, they can become a potent force for change. Findings through secondary data showed that although women have an important role in rural development in Iran, but there are some problem faced by women farmers. The finding can assist the local organizations and community developers for remove this problem**.**

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Key words: women farmers, rural development, agricultural development

Introduction

The subject of"women and development" has invited a good deal of attention, especially in the case of rural women, in recent years and rightly so. Women constitute about 50 percent of the world's population and one-third of its labor force (Reddy & Rani, 1982). Women play a very important role not only in running the household but also the community development. It is argued that the rural women in Iran have been actively involved in agriculture (Jamali, 2009). Women's work in agriculture has become more visible over the last few decades as women farmers become more involved in agricultural activities, increasingly assuming the responsibility for household survival and responding to economic opportunities in agricultural production. This has made rural development considerably dependent on the capacity enhancement of women farmers as they increasingly provide a vital contribution to the development of rural communities. Despite the growing importance of the women farmers' role in agriculture, as well as in rural development, the lack of access to and control over productive resources deprives them of opportunities for their own capacity building and improved quality of life. In addition, the long tradition of gender gap and inequalities seriously restricts women's right to legal, institutional and policy support services. They are disadvantaged through limited access to land, credit, technology and information, and especially in the remuneration of their activities resulting to a wide underestimation of women's economic contributions. Also, there are very few programs for enhancing women farmers' welfare and their empowerment under a new rural environment (ASIADHRRA, 2007).

Methodology

The research was performed as a qualitative library in which the researcher had to refer to relevant and related sources. Sources that we used to collect needed information about Iran in order to write this article were the, rural cooperatives, Ministry of agriculture State Planning Organization, official websites of agriculture in Iran, as well as relevant literature and articles about the rural development. Likewise, I have used a number of articles and official websites of the various world known organizations.

Rural Development

The concept of rural development has changed significantly during the last 3 decades (Harris, 1982). Until the 1970s, rural development was synonymous with agricultural development and, hence, focused on increasing agricultural production (Fernando, 2008). Rural development in general is used to denote the actions and initiatives taken to improve the standard of living in non-urban neighborhoods, countryside, and remote villages. These communities can be exemplified with a low ratio of inhabitants to open space. Agricultural activities may be prominent in this case whereas economic activities would relate to the primary sector, production of foodstuffs and raw materials (Wikipedia, 2010).

Rural development is a more specific concept than the concept of community development. In broad terms, rural development is about improving the quality of life of all members of rural community (Figure 1).Rural development covers three different but interrelated dimensions. The first is the economic dimension that encompasses providing both capacity and opportunities for the poor and low-income rural

households (UNDP, 2005). The economic dimension also includes measures to reduce intra- and inter-sectoral income inequalities to reasonable levels. Second is the social dimension of supporting social development of poor and low-income households and disadvantaged groups, eliminating inequalities in social indicators, promoting gender equality and women's empowerment, and providing social safety nets for vulnerable groups. Third is the political dimension of improving opportunities for the poor and low-income people in rural areas, including women and ethnic minorities, to effectively and equally participate in the political processes at the village level and beyond and outside rural areas (Fernando, 2008).

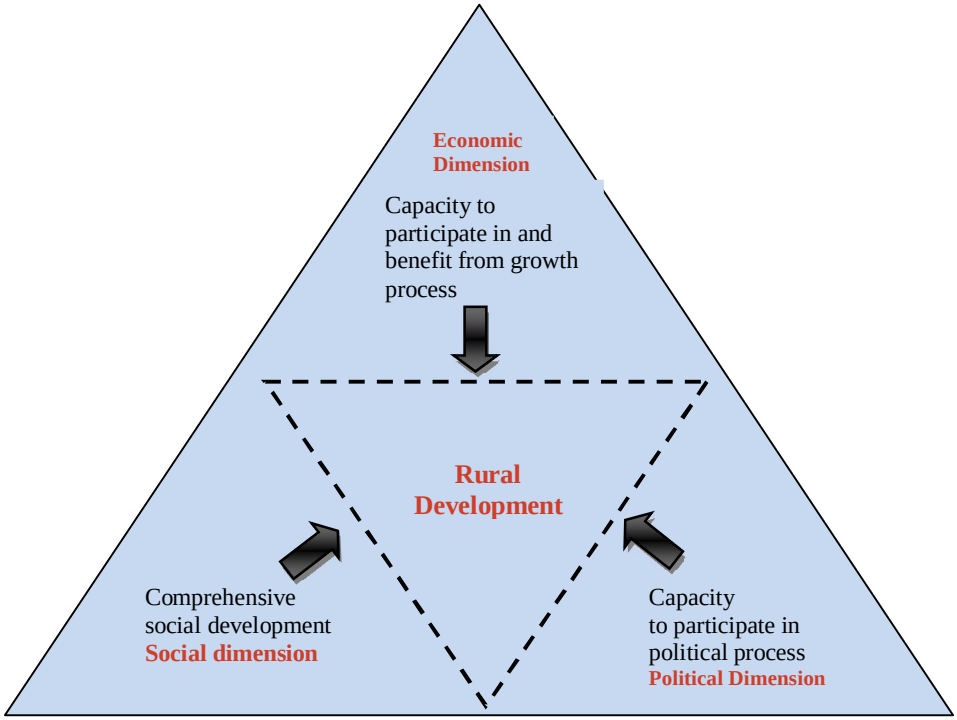

Figure 1: Three Dimensions of Inclusive Rural Development. Adapted from Fernando (2008)

Women's Empowerment for Rural Development

The meaning of "empowerment" turns on what we mean by power. Most social scientists use a definition of power something like Max Weber's (1992): 'power is the probability that one actor within a social relationship will be in a position to carry out his own will despite resistance , regardless of the basis on which this probability rests (Presser & Sen, 2003). The World Bank has identified women's empowerment as one of the key constituent elements of poverty reduction, and as a primary rural development goal. The Bank has also made gender mainstreaming a priority in development assistance, and is in the process of implementing an ambitious strategy to this effect. The enhancement of women's empowerment is the main rural development goal (Malhotra, Schuler, & Boender, 2002). Where agriculture is a primary occupation, women work to produce food for their families and where non-agricultural employment is not available, they may become informally self-employed, producing goods and services, within their capacity, to be marketed locally. Empowering women through education significantly impacts their survival rate and that of their children as well as the overall health and economic welfare of their families(UNICEF, 2010). Because of the empowerment of women, the elimination of gender discrimination and the creation of a balance of power between men and women, will not only be beneficial to women, but society as a whole shall benefit politically, economically and culturally (Allahdadi, 2010).

I propose the model of women's empowerment in Figure 2. It states that the macro social factors of laws, organizational rules and norms affect women's access to economic resources and their subjective states of self-efficacy and entitlement (Presser & Sen, 2003).

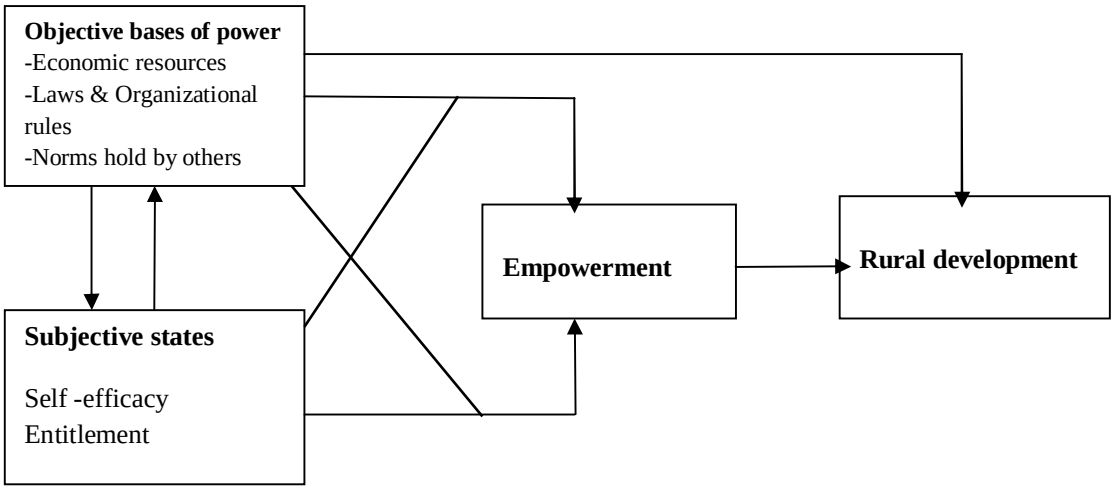

Figure 2: Model of women's' empowerment. Adapted from (Presser & Sen, 2003)

Problem Faced by Women Farmers in Iran

Women's contribution to local and community development is significant, but rural women everywhere are in a minority in decision-making and planning, particularly at regional and national levels. This is in part due to women's multiple roles and workload, but is also due to the persistence of traditional views about women's and men's roles in society (European Commission Directorate-General for Agriculture, 2000). Many factors contribute to generate some problems for women in rural agricultural development. The loss of lands, waters and forests experienced by local peoples is especially deepening the poverty of local women while increasing their domestic load and subsistence responsibilities. Since many indigenous women are also illiterate or have a low educational level, they continue to be excluded from job opportunities and rural management roles. Changes in traditional social, cultural and political institutions and practices have led to a loss of rules and codes of behavior that have long been instruments in ensuring gender-sensitive structures. Rural women in many developing countries face numerous barriers in their access to education, health care, sanitation and other basic services, and are excluded from decision-making on programs to meet these needs and entitlements.

Factors that effect on women empowerment in rural development can be include:

- Tradition and culture. Although this is the most commonly cited impeding factor, it should be clear that culture is not static
- Women have limited mobility and high levels of illiteracy.
- Insufficient financial resources. The resources allocated for the provision of information, services and resources to women often cannot support separate women's development components.
- Equipped local agricultural institutions. Agricultural institutions are not set up to meet the challenges of reaching out and working with rural women.
- Unsuitable administrative and financial procedures. The implementing agency's procedures are generally not adapted to the requirements of working with rural women and to a timely response to their needs.
- Lack of specific focus on women's development in extension agents' job descriptions. The job descriptions of extension agents generally do not support working with women and facilitating women's awareness of their own potential, ability negotiate their own needs, and capacity to access and manage new resources.

- Insufficient understanding of development and gender. There is a need to strengthen the understanding of development and gender, and the communication, planning and monitoring skills not only of the field agents working with women, but also of the management staff supervising and advising these field officers.
- Undervaluing of women's contribution to development. Activities targeted at women are often assimilated with domestic activities and their added value undermined, thus leading to an underestimation of women's potential contribution to development (IFAD, 2010).

The common problem among rural women in Iran can categorized into four major categories; including: economic, program, political and socio culture issues

1) Economic issues: such as rural women's lack of title to productive assets and access to "inputs" (land, credit, water, fertilizer, seeds, information, technology, training, etc.) and markets; the increasing drudgery and time spent by women in agricultural activities which is not compensated by increases in value added; and the limited availability and/or relevance of technology and other aids for women.

2) Political issues: such as institutional barriers to women's political participation and organization (patriarchy, non-organization of women, rural isolation).

3) Socio-cultural issues: such as low status and disadvantaged position of women resulting in lower education; little access to training; non-participation in decision making; lower income; poor nutrition and health; few property rights.

4) Planning issues: such as inadequate gender-differentiated and disaggregated data, as well as data gaps with regard to rural women, which results in overlooking gender issues for planning; and lack of appropriate methodologies that recognize and value women's contribution, actual and potential, to productive activities resulting in women's marginalization in projects and programs.

Rural women in their dual roles as producers in the farm and the home and as caregivers need appropriate technologies to ease their work stress and to improve productivity. In developing countries, technology development and extension programs have not been responsive to household drudgery associated with different production activities undertaken by women. Hence, rural women's demand for technology that improves their productivity while reducing drudgery must be recognized. Most countries still lack adequate provision for women to hold land rights independently of their husbands or male relatives. Land ownership in rural areas determines the asset for production as well as access to credit and agricultural support services and the social power to negotiate for resources and membership in decision-making agencies. Hence, rural women must be empowered with legal and institutional measures to secure land and other resources. (ASIADHRRA, 2007).

Conclusion

Agriculture is certainly a major contributor to rural development in many countries. It is one of the most important economic sectors in Iran. Rural women are major contributors in agriculture. However, the women's status is low by all social, economic, and political indicators. Without education, it is more difficult for women to move out of poverty and they enter into a vicious circle of reduced employment opportunities and occupational mobility, lower income, early marriage, poor child health care and increasing fertility. In other words, without women's empowerment the rural development goals cannot be achieve.

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Simultaneous diffusion of Cr-Si on Ni-Base super alloy using pure Cr and Si by pack cementation method

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Abstract: Pure Cr and Si powders were used to produce Cr-Si coatings by Simultaneously diffusion of these elements on Ni-base Super alloy. A mixture of elemental Cr and Si powders (as Cr, Si sources) was used with (NaCl-NaF) or (NaCl-NaF-NH_4Cl) mixed activators were applied. The results of this study indicated that for co diffusion of these elements, Si content must be 0.1 Cr content in the pack mixture. Using 95%NaCl-5%NaF mixed activator was produced porous Cr-Si coatings, but by addition of 1% NH_4Cl to pack mixture, porosity of Cr-Si coating was eliminated. Increasing of (NaCl-NaF) content was leaded to increase depth of Si diffusion into the surface.
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Key words: Simultaneous diffusion, pack cementation, pure elements, mixed activator, Super alloy.

1. Introduction

To protect the hot components in land-based gas turbines, used in power generation, a wide range of coatings are applied to these parts [1]. One of them is diffusion coating in which elements are diffused into the surface of components. A common way to produce diffusion coatings is through pack-cementation process due to its simple and cost effective advantages [2].
Alloying elements used in the diffusion coatings include Al, Cr, Si, or combinations of these elements [3-6]. Cr-Si diffusion coatings have been produced by following methods:

1- Simultaneous diffusion of Cr and Si by pack-cementation [7-9]
2- Two step pack-cementation process in which Cr, diffused in substrate prior to diffusion of Si

It would be technically and economically more efficient if Cr and Si could be deposited simultaneously in a single step [4]. Therefore, there is a strong technical incentive to further develop the pack-cementation process so that multiple elements such as Cr, Si and etc can be co-diffused to produce high temperature oxidation and corrosion protective coatings. For Simultaneous diffusion of Cr and Si, the vapor pressure of Cr and Si halides (generated in the pack mixture trough reactions between these elements with activators) should be comparable. Many researches have been made to identify suitable conditions for co-diffusion of Cr with Si using Si-Cr master alloys in the pack mixture [10].
In the present paper, studies on the feasibility of co-diffusion of Cr with Si, using pure Cr and Si. The cross section of produced coatings is evaluated by electron microscope (SEM) and distribution of elements determined by line-scan, mapping and quantitative analysis with energy dispersive x-ray spectroscopy (EDS).

2. Experimental Procedures

The substrate used in this investigation was Nickel- base super alloy INC738 in the ingot form with the composition, shown in Table 1.

Specimens in dimension of Ø15×20 mm were cut from ingot, and then grounded with 120-300-600 grit SiC paper, cleaned in acetone and dried. The samples were placed in a 10×10×10cm Coat box, made of stainless steel and filled with pack mixture, containing elemental Si and Cr, an activator salt(s) and inert alumina-filler. The composition of pack mixtures has been shown in Table 2.

Semi sealed coat boxes were placed into a retort, which set up in an electric furnace and retort purged with argon in a flow rate of 10 $litmin^{-1}$ from the onset of furnace heat up to provide an inert environment. After completion

of coating time, coat boxes were allowed to furnace cool and were removed and discharged. Then, the coated samples were cleaned, mounted and polished. SEM with an EDS was used to determine the structure, thickness and concentration profiles across the coating cross-section.

Table 1 - chemical composition of IN738LC

Element Al	TI	Cr	Co	Mo	Ta	Nb	Zr	Ni	
Wt%	3.5	3.42	15.8	8.5	1.6	1.81	0.86	0.05	bal

Table 2 - chemical composition of pack mixtures

pack	Composition
A	23%Cr -10%Si-2 %(95%NaCl-5%NaF)-65%Al_2O_3(wt%)
B	23%Cr -5%Si -2% (95%NaCl-5%NaF)-70%Al_2O_3(wt%)
C	23%Cr-2.3%Si-2%(95%NaCl-5%NaF)- 72.7%Al_2O_3 (wt%)
D	23%Cr-2.3%Si-2%(95%NaCl-5%NaF)-1.5%NH_4Cl-71.2% Al_2O_3 (wt%)

Semi sealed Coat boxes were placed into a retort, which set up in an electric furnace and retort purged with argon in a flow rate of 10 litmin^{-1} from the onset of furnace heat up to provide an inert environment. After completion of coating time, coat boxes were allowed to furnace cool and were removed and discharged. Then, the coated samples were cleaned, mounted and polished. SEM with an EDS was used to determine the structure, thickness and concentration profiles across the coating cross-section.

3. Results and Discussions

According to thermo dynamical calculations, volatile halides of Si are more stable than those of Cr. Therefore, Cr-reach pack mixtures must be used to generate comparable Cr and Si halides partial vapor pressures to co-deposition and diffusion of these elements [10, 11].

Fig. 1 shows the microstructure and line-scan analysis of the specimen coated at 1100 ºC for 2 h in pack (A) with composition, listed in table 2.

According to Fig.1 a thick coating (with 325µm thickness) enriched with Si, is produced at mentioned condition. it means that, the volatile halides of Si , which generated in pack (A) , had higher vapor pressure than those for Cr. Fig.2 shows the microstructure and line-scan analysis of the specimen coated at1100 ºC for 2 h in pack (B) with composition, listed in table 2. This coating also consisted of Si and indicated that Cr did not co diffuse with Si.

Fig.3 shows the microstructure and line-scan analysis of the specimen coated at 1100 ºC for 2 h in pack (C) with composition, listed in table 2. Fig. 3 shows that co-deposition of Cr and Si, achieved in a pack mixture contained elemental Cr and Si (which the Cr content is tenfold of Si) and (95% NaCl-5% NaF) mixed activator at 1100 ˚C and 2 h. The thickness of coating is low (~14µm) and has porosity.

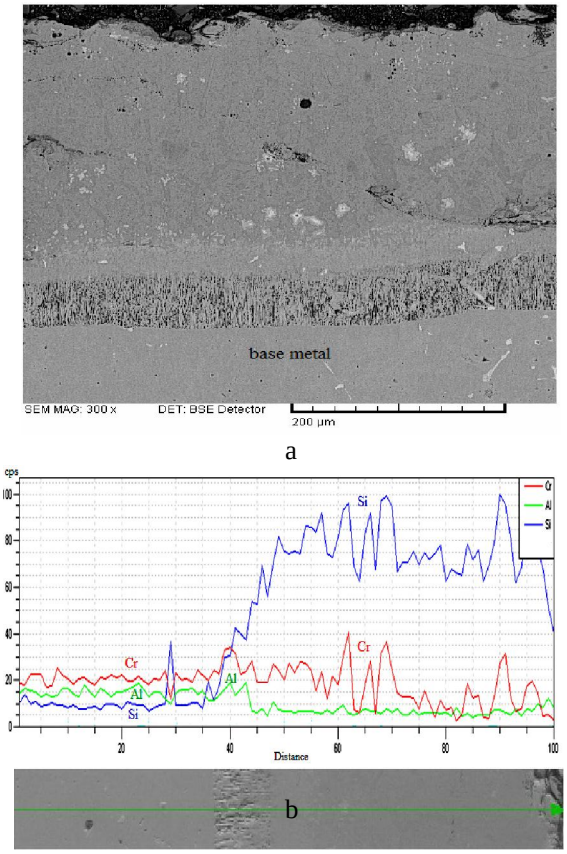
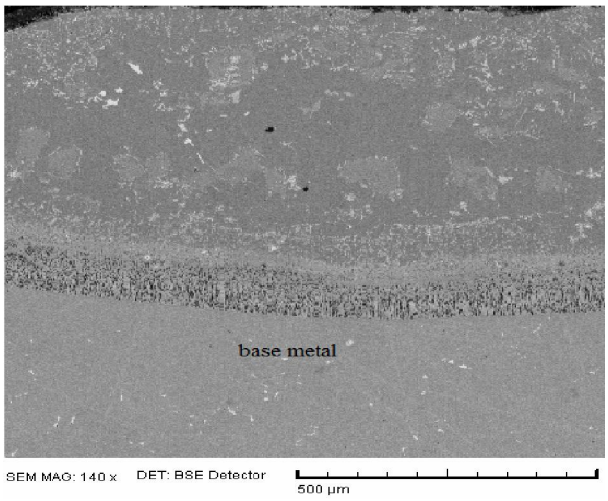
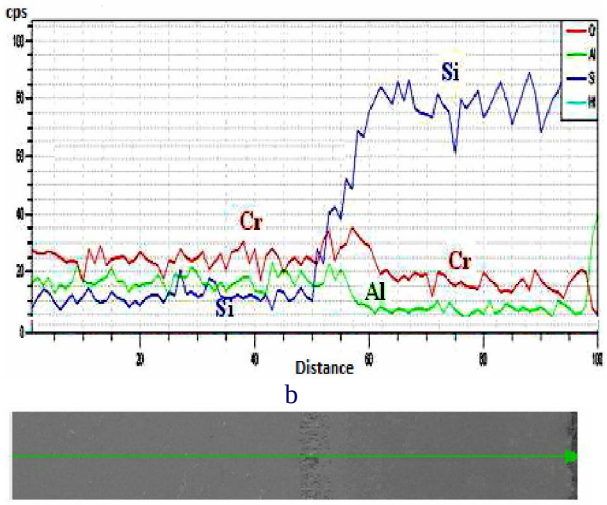


Fig.1 a) microstructure and b) line-scan analysis of the specimen coated at 1100 ºC for 2 h in pack (A)



a



b

Fig.2 a) microstructure and b) line-scan analysis of the specimen coated at 1100 ºC for 2 h in pack (B)

Fig. 4 shows the microstructure and line-scan analysis of the specimen coated at 1100 ºC for 3 h, in pack (B) with composition, which is given in table 2. This figure also shows that the produced coating at this condition , is without any porosity and has a total thickness of a bout 40 µm. In consequence, the addition of NH4Cl to (NaCl-NaF) as an activator was leaded to increase of coating thickness and elimination of porosity.

NH_4Cl is an unstable activator and decomposed as follows [12]:

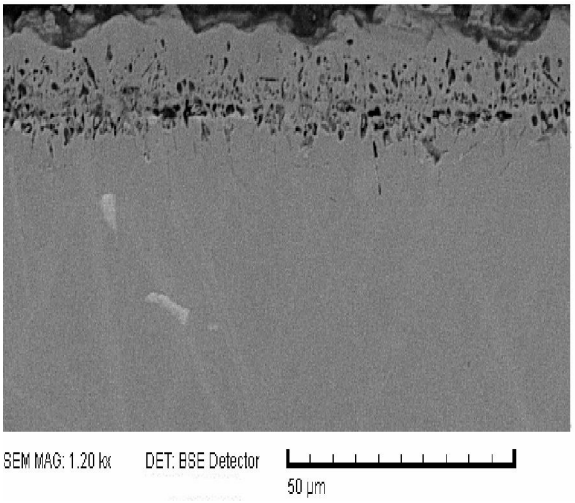
$$NH_4Cl_{(g)} \rightarrow {}^{1}/_{2}N_{2(g)} + {}^{3}/_{2}H_{2(g)} + HCl_{(g)} \quad (1)$$

Dissociated HCl from NH_4Cl could increase the kinetic of volatile halides generation reactions (reactions of Cr and Si with activators) in the pack mixture. Also, dissociated H_2 could change the reactions of volatile halides in the substrate surface from displacement or dissociation to reduction type as follows [13]:

$$Cr_xCl_{y(g)} + {}^{y}/_{2}H_{2(g)} \rightarrow yHCl_{(g)} + xCr_{(s)} \quad (2)$$

$$Si_xCl_y + {}^{y}/_{2}H_{2\,(g)} \rightarrow yHCl(g) + xSi(s) \quad (3)$$

Then Pack (B) was provided suitable condition for Cr and Si co-deposition as mentioned a bove. Effect of increasing (NaCl-NaF) content in the pack mixture, on the coating thickness is shown in fig 5. It is illustrated that in 3.5% (95%NaCl-5%NaF) the thickness of coating increases rapidly. It is due to increasing in vapor pressure of Si and Cr fluorides in the pack mixture, which leaded to more transportation of Si and Cr from the pack mixture to surface of the sample.



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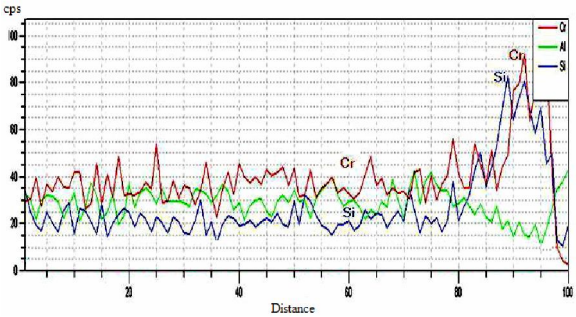

b

Fig.3 a) microstructure and b) line-scan analysis of the specimen coated at 1100 ºC for 2 h in pack (C)

4. Conclusions:

The evidences from experiments showed that pure Cr and Si can be applied to simultaneous diffusion of Cr, Si by pack-cementation process. Si content of pack mixture should be 0.1 Cr content for co diffusion of them. A mixture of 1%NH_4Cl –2% (95%NaCl-5%NaF) is the best activator to produce Cr-Si coating with enough thickness and without porosity. Increasing of (95%NaCl-5%NaF) content in the pack mixture was leaded to increase thickness of coating. In 3.5% (95%NaCl-5%NaF) thickness of coating rapidly increases.

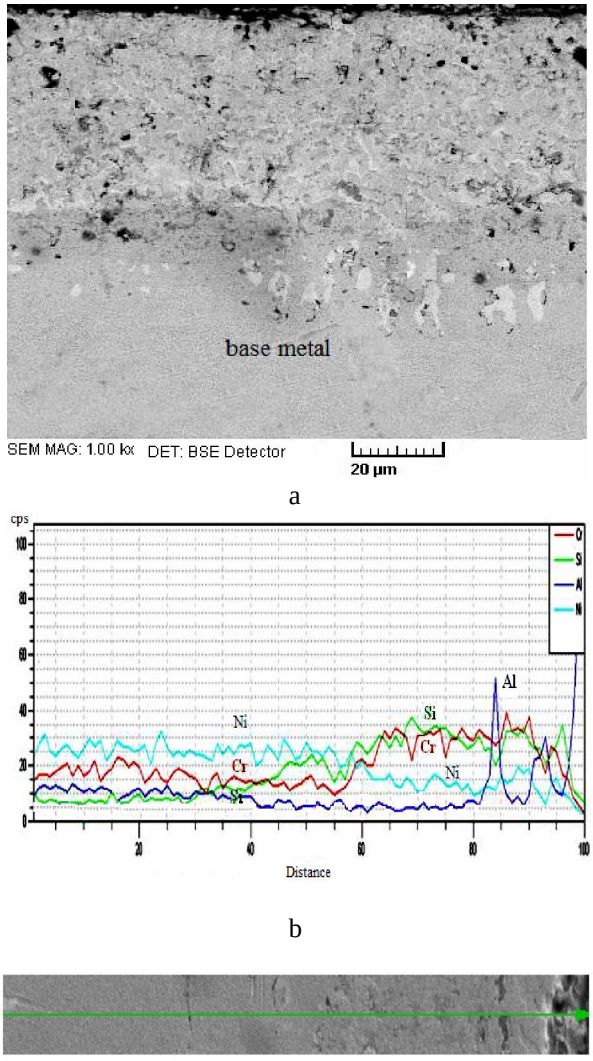

Fig.4 a) microstructure and b) line-scan analysis of the specimen coated at 1100 ºC for 2 h in pack (D)

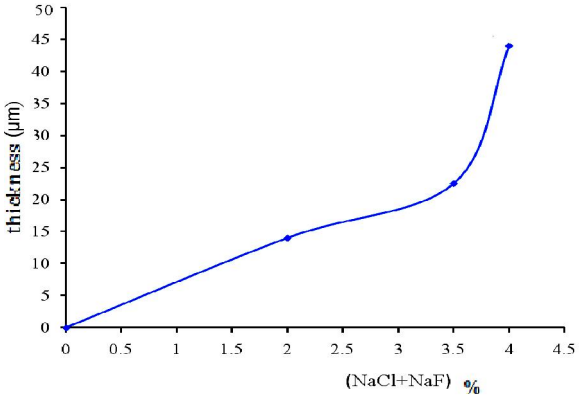

Fig.5. The effect of increasing (95% NaCl-5%NaF) content in the pack mixture on coating thickness.

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Effect of some Strains of Probiotic Bacteria against Toxicity Induced by Aflatoxins *in vivo*

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Abstract: Aflatoxins are highly toxic, mutagenic, teratogenic and carcinogenic compounds produced by some species of Aspergillus, especially *A. flavus* and *A. parasiticus.* This study was conducted investigate the effect of some strains of probiotic bacteria against toxicity induced by contaminated diet with aflatoxins in male rats. Animals were divided into 6 equal groups each group contains 7 rats. The first group received a basal diet and served as negative control, the second group received basal diet supplemented with strain 1 of probiotic bacteria (*Bifidobacterium bifidum),* the third group received basal diet supplemented with strain 2 of probiotic bacteria *(Lactobacillus acidophilus),* the fourth group received basal diet supplemented with 1.34ppm aflatoxins contaminated peanut as positive control group. The other two groups received basal diet supplemented with 1.34ppm aflatoxins contaminated peanut plus strain 1and strain 2 probiotic bacteria for 6 weeks. Results revealed that positive control gave a very significant increased in alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline Phosphatase (ALP) activities, creatinine and urea; while decreased total protein (TP), albumin and globulin indicating the toxicity of aflatoxin on both liver and kidney functions. However probiotic strains supplemented to aflatoxins treated group revealed a significantly alleviated TP, albumin and globulin depletion in serum with an elevation of ALT, AST, ALP, creatinine and urea levels. Results also showed that the group received basal diet supplemented with strain 1 (*Bifidobacterium bifidum)* and with strain 2 *(Lactobacillus acidophilus)* showed significant beneficial health effects. It was noticed that the group received *Lactobacillus acidophilus* showed better results than *Bifidobacterium bifidum.* Results indicated also that the protective action of probiotic strains as a potential protective agent against aflatoxin toxicity as well as their beneficial health effects and may thereby offered an effective dietary approach to decrease the risk of occurrence of liver, kidney function and occurrence of cancer which may be due the ability of probiotic strains to bind with aflatoxins, reduced their uptake, and protected against both memberane and DNA damage. The study revealed also that probiotics can also provide benefits by modulating immune functions.

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Key words, Mycotoxin, Aflatoxin, Peanut, Toxicity, Probiotic bacteria

1. Introduction:

Aflatoxins (AFs) are highly toxic secondary metabolites produced by the species of Aspergillus, especially *A. flavus* and *A. parasiticus*. These fungi can grow on a wide variety of foods and feeds under favorable temperature and humidity. Contamination by aflatoxins can take place at any point along the food chain from the field, harvest, handling, shipment and storage (Giray *et al*., 2007).

Aflatoxins (AFS) have been found to contaminate a wide variety of important agricultural products world-wide such as corn, wheat, rice, spices, dried fruits, and nuts. These compounds can enter the food chain mainly by ingestion through the dietary channel of humans and animals (Aycicek *et al*., 2005). Concerns related to the negative health impacts of AFs have lead to the investigation of strategies to prevent their formation in foods, as well as, to eliminate, inactivate or reduce the bioavailability of these toxins in contaminated products. Techniques to eliminate, inactivate or reduce the bioavailability of AFs include physical, chemical, and biological methods. These processes have at least two drawbacks; high cost of removing and disposing of the contaminated materials and difficulty achieving complete removal contaminated materials without wasting significant portions of uncontaminated product (Méndez-Albores *et al*., 2007). Limitations such as loss of product nutritional and sensory qualities, as well as, the expensive equipment required for these techniques have encouraged the recent emphasis on biological methods (Teniola *et al.*, 2005).

Lactic acid bacteria (LAB) and bifidobacteria, due in large part to their generally recognized as safe (GRAS) status and use as probiotics, are of particular interest for reducing the bioavailability of AFs. A number of studies have

screened these microorganisms for its ability to bind to AFs and have reported a wide range of genus, species and strain specific binding capacities. Most of previous studies focused on the *ex vivo* studies but little studies focused on *in vivo* studies (Hwang *et al*., 2005; Zinedine *et al*., 2005; Shahin, 2007; Dalié, 2010).

The whole concept of probiotics is not new, and in fact they have been consumed by human beings in the form of fermented foods, for thousands of years (Kopp-Hoolihan, 2001). Their health benefit has also been long known, in early ages being reported that fermented milk could cure some disorders of the digestive system (Lourens- Hattingh & Viljoen, 2001). Today it is accepted that daily intake of these probiotics contributes to improving and maintaining well balanced intestinal flora, and prevents gastrointestinal disorders (Lavermicocca, 2006). Various species of genera Lactobacillus and Bifidobacterium mainly and some other species of micro-organisms have been used as probiotics over the years (Boyle & Tang, 2006). Different strains of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* could be considered as the main microbial species that have been use as probiotics (Shahin, 2007; Ranadheera *et al*., 2010). This study aimed to investigate effect of two strains of probiotic bacteria (*Bifidobacterium bifidum*, and *Lactobacillus acidophilus*) against toxicity induced by aflatoxins *in vivo*.

2. Materials and methods

Peanut Samples

Six kilograms of peanut were obtained from Egyptian local market.

Chemicals

Chemicals used in this study were obtained from Sigma Chemical Company (St. Louis, USA).

Media

MRS Broth and MRS Agar were obtained from Oxoid Ltd., Wade Road, Basingstoke, U.K.

Diagnostic Kits

Commercial kits were purchased from Bio Merieux Company (L'Etoile /France) and from Eagle Diagnostics (Dollas, TX, USA).

Probiotic Bacteria

Two strains of probiotic bacteria were used in this study. One of them was obtained from local market and the other has been prepared in vitro.

1- Strain 1 *Bifidobacterium bifidum* was obtained from Chr. Hansen's Lab, Denmark. The *Bifidobacterium bifidum* strain proved to have probiotic properties.

2-Strain 2 (*Lactobacillus acidophilus*) as Pharmaceutical product manufactured by Ramada (The tenth of Ramadan) CO. 6 of October city ARE (Arab Republic of Egypt) under license of Axcan pharma .S.A. France as a powder.

Animals

Forty two male adult Albino rats (Sprague-Dowley strain) with an average weight 130 ± 10g were obtained from animal house of NRC. Rats were divided into 6 groups (each group 7 rats) and housed in galvanized metal cages. Food and water were supplied ad libtum for 6 weeks. All rats were adapted for three days on the control diet before the beginning of the experiment.

Detection of Aflatoxins

Aflatoxins were detected in peanut sample according to A.O.A.C (1995).

Activation of Tested Strains

Bifidobacterium bifidum was enumerated according to DeMan, *et al*., (1960) using modified MRS Broth (Oxoid) supplemented with 0.05% L.cysteine HCL (Merck, Germany). *Lactobacillus acidophilus* was activated in MRS Broth both and anaerobically incubated at 37ºC for 24h.

Preparation of Bacterial Strains

Strain1 (*Bifidobacteria)* was prepared at Food Toxicology and Contaminants, NRC in vitro as follow: 5.0 ml of the activated tested bacteria was added to 500 ml of modified MRS Broth. After that it was incubated at the optimic temperature (37 °C under anaerobic conditions) for 24 hrs then it was cinterifugated at (3000 x g, 4°C, 20 min) to harvest the cells. Dehydration was obtained by addition 50 g of defatted soy protein (soy protein without fat) to cells in big Petri dishes and the cells were incubated under vacuum incubator at 40^{o}C overnight until it seemed like as thin slice or skins. The viability of the cells was tested on MRS agar plates then, the strain was chopped and made as a powder containing 10^{9} of bacteria/g.

The strain 2 (*Lactobacillus acidophilus*) powder was obtained as Pharmaceutical product containing 10^{9} of bacteria/g. The bucket contains 6 sachets.

Experimental Animals

Diet Preparation

Basal diet was prepared according to the method described by Campbell, (1963) on diet bases: Protein (12%), fat (10%), salt mixture (4%) vitamin mixture (1%), Choline chloride (0.25%), and cellulose (5%) corn starch (up to 100). The vitamin mixture was prepared according to Campbell, (1963). The salt mixture was prepared according to Hegsted *et al*., (1941).

Experimental Design

The forty two rats were divided to 6 equal groups as following:

Group 1 (G1): fed on basal diet (negative control); Group 2 (G2): fed on basal diet + strain 1 of probiotic bacteria (*Bifidobacterium bifidum).*

Group 3 (G3): fed on basal diet + strain 2 of probiotic bacteria *(L. acidophilus).*

Group 4 (G4): fed on 10% natural contaminated peanut with aflatoxins (Positive control).

Group 5 (G5): fed on 10 % contaminated peanut with aflatoxin + strain 1 of probiotic bacteria; Group 6 (G6): fed on 10 % contaminated food of aflatoxin + strain 2 of probiotic bacteria.

Biochemical Analyses

At the end of the experiment rats were fasted overnight (about 12 hrs) and anesthetized with diethyl ether. Blood samples were collected in clean dry centrifuge tubes from hepatic portal vein. All blood samples were centrifuged for 15 minutes at 3000 rpm to separate the serum. Serum was kept frozen at (-20°C) till analysis (3-5 days). According to (Jacobs *et al*., 2001).The toxicity of aflatoxins and the protective effect of Probiotic bacteria against aflatoxins toxicity were evaluated by determination of serum ALT and AST activities according to Henry (1974) and Yound (1975), respectively. Enzymatic calorimetric determination of serum alkaline Phosphatase was carried out according to Belfield and Goldberg (1971) as liver function tests.

Determination of Serum Total Protein (T.P) according to (Gornal *et al*,.1949) and Serum albumin was determined as g/dl according to the method described by Weiss man *et al*., (1950).

Serum Globulin was calculated as g/dl according to Chary and Sharma, (2004).

Serum A/G Ratio was calculated according to Sirvastava *et al*., (2002).The principle use of urea determination according to Carawy, (1955) .Creatinine was determined according to Larsen, (1972). These blood serum parameters were measured colorimetricaly using kits purchased from Bio Merieux Company (L'Etoile /France) and from Eagle Diagnostics (Dollas, TX, USA) and were measured using a spectrophotometer U.V/visible Jenway 1640.

Histopathological Examination:

At the end of the experiment, rats from each group were anesthetized with light ether then sacrificed by decapitation. After animal dissection, the liver & kidneys, were removed, thoroughly washed with a physiological saline (0.9% NaCl) solution and blotted on filter paper. Organs specimens were rapidly fixed in Bruin's solution for 4h then retained in 70% alcohol until processing. The fixed specimens were processed using a conventional paraffin embedding technique. From the prepared paraffin blocks,5 mm thick sections were obtained and stained with hematoxylin and eosin (H&E) for light microscopic examination (Culling, 1983). Specimens from liver and kidney were collected after kept in formalin then embedded in paraffin 4/6 thin sections were prepared and stained with hematoxylin and eosin according to Carleton, (1978).

Statistical Analysis

Statistical analysis was performed by using computer program COSTATE and compared with each other using the suitable tests. We used one way ANOVA ((Armitage and berry (1987)

Results are reported as

1-mean ± SD

2- P value differences with $p \leq 0.05$ were considered to be significant $p<0.05$ very significant

3. Results and Discussion:

Levels of aflatoxins in peanut and contaminated diet

As shown in table (1); levels of aflatoxins B1, B2, G1, G2 and total aflatoxins were 3.3±0.8, 1.0±0.2, 7.0±1.7, 2.1±0.5 and13±3.3 µg/kg peanut respectively. The levels of aflatoxins in basal diet supplemented with 10% contaminated peanut were 0.33, 0.1, 0.02, 0.7, 0.21 and 1.34 µg/kg for aflatoxin B1, B2, G1, G2 and total aflatoxins respectively. These results agree with Ayesh and Ismail, (2001) who screened the toxigenic fungi and aflatoxins production in different variety of peanut (Early Bunch, Gregory, Romy and NC) before and during the different processing stages as well as during storage and Sultan (2004) who reported that the aflatoxins in naturally contaminated peanut seed reached to 26.7ppb.

The knowledge that mycotoxins can have serious effects on humans and animals has led many countries to establish maximum tolerated level (MTL) on mycotoxins in foodstuffs and feedstuffs in the last decades to safeguard the health of humans, as well as the economical interests of producers and

traders. Currently, worldwide range of limits for AFB1 and total AF (AFT) are 1-20 ng/g and 0–35 ng/g, respectively (FAO, 2004).

Table 1: Levels (µg/Kg) of Aflatoxins in Peanut

Aflatoxin	Amounts (µg/Kg)
AFB1	3.3±0.8
AFB2	1.0±0.2
AFG1	7.0±1.7
AFG2	2.1±0.5
T AF	13.4±3.3

Effect of probiotic bacteria on body weight gain, food intake and feed efficiency ratio

As shown in Fig 1, the group received contaminated peanut with aflatoxins showed significantly lower in BWG, FI and FER ($p<0.05$) compared with basal diet which may be due to the loss of animals appetite caused by aflatoxin. Similar results were obtained by Parlat *et al.*, (1999) who found that BWG and feed conversion rate (FCR) were decreased significantly by AFB1 treatment compared with control and Denli *et al.*, (2003) who reported that, aflatoxin B1 (AFB1) caused non significant reduction in Body Weight Gain (BWG) and (FCR) by 9.3 and 7.6 % respectively.

The decreased in BWG, FI and FER were significantly ($P \leq 0.05$) improved (by probiotic bacteria supplemented to aflatoxin treated group. In addition BWG in the group received probiotic bacteria strain2 (*lactobacillus acidophilus)* was significantly ($P \leq 0.05$) higher compared with negative control and FI and FER were around negative control. These results indicated the health benefit and the effect of probiotic bacteria against toxicity induced by aflatoxins. This occurred as a result of decreased uptake of toxins caused by *Bifidobacteria* which lead to increasing FER and BWG (Solga, 2003).

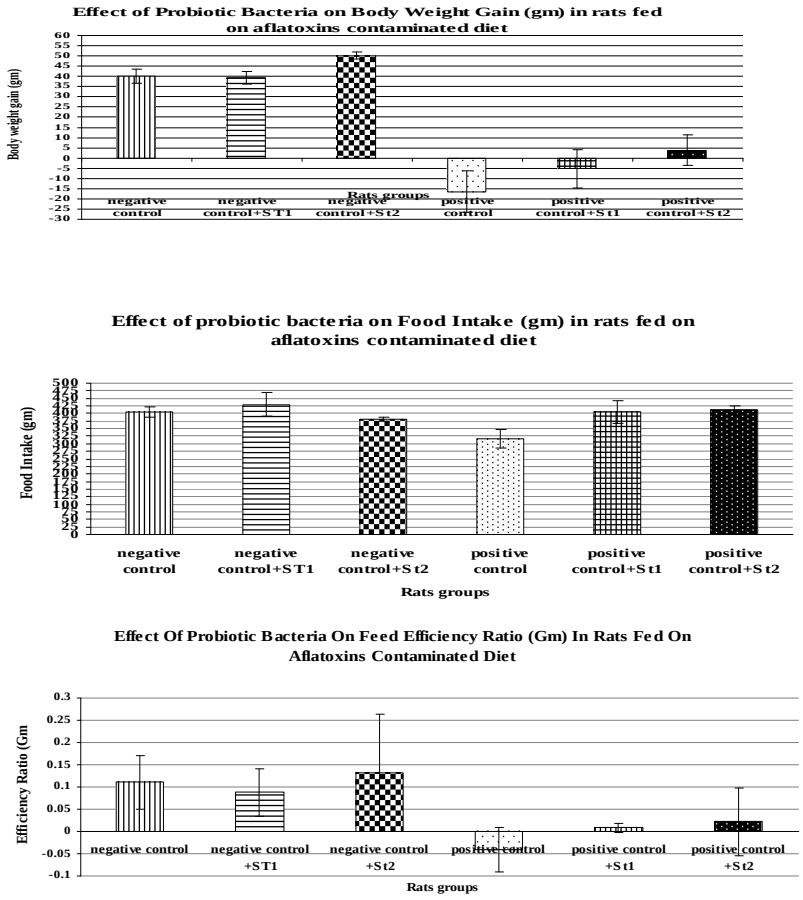

Fig.1: Effect of probiotic bacteria on body weight gain, food intake and feed efficiency Ratio

Effect of probiotic bacteria on liver functions in rats fed aflatoxins contaminated diet

The results in Fig 2 (A,B,C) showed that aflatoxins treatment caused very significant ($P < 0.05$) increased on serum liver function enzymes ALT, AST and ALP. The affected liver functions by aflatoxins were achieved by Zohair (1996), Denli *et al*., (2003) and Kermanshahi, *et al*., (2007) who demonstrated that feeding aflatoxin B1 (AFB1) may have some adverse effects on the liver and brain of broilers. Probiotic strains *Bifidobacterium bifidum* and *lactobacillus acidophilus* supplemented to aflatoxins treated group showed a significant ($P < 0.05$) improved in liver functions. It was also noticed that *lactobacillus acidophilus* is better than *Bifidobacterium bifidum* strain. This occurred as a result of the ability of microorganisms to bind aflatoxins and have reported a wide range of genus, species and strain specific binding capacities (Peltonen et al., 2000; Peltonen *et al*., 2001). In addition; Peltonen *et al*., (2000) assessed the ability of six probiotic bacteria to bind a common food carcinogen, aflatoxin B1 in vitro. The studied strains included *Lactobacillus* strains and one *Bifidobacterium* strain. The aflatoxin-binding capacity of the strains was found to range from 5.8 to 31.3%. Although the extent of binding varies depending on the bacterial strain used, the data may explain some of the antimutagenic and anticarcinogenic effects of probiotic micro-organisms.

In vivo study, EL-Nezami *et al*., (2006) concluded that probiotic supplement reduced the biologically effective dose of aflatoxin exposure and may thereby offer an effective dietary approach to decrease the risk of liver cancer. Also Gratz *et al*., (2007) found that probiotics, especially GG are able to bind AFB1 under in vivo conditions in rats and intestinal cells.

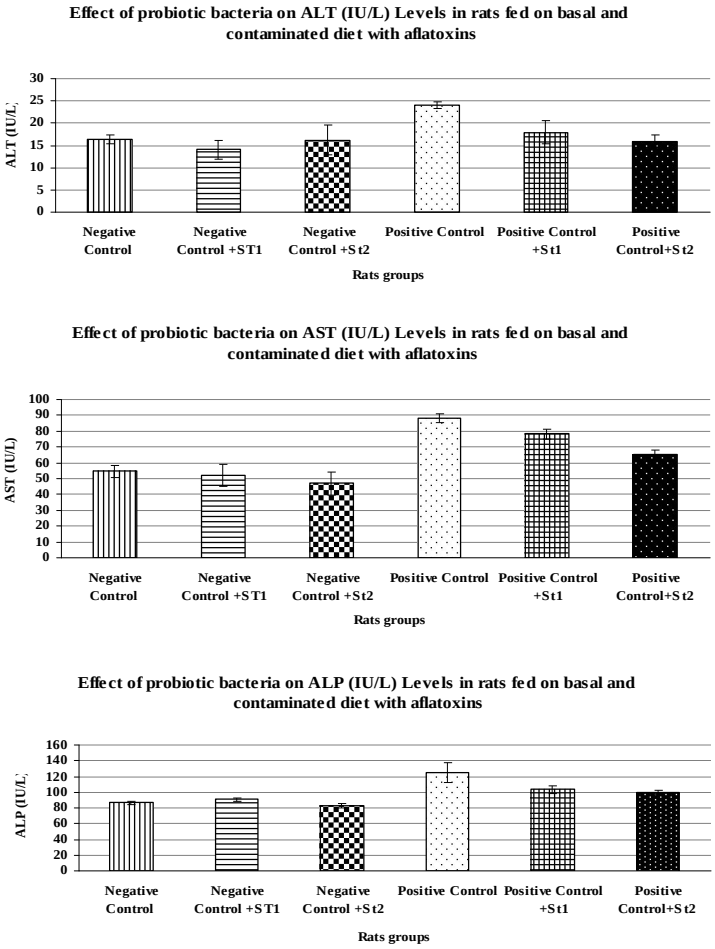

Fig.2: Effect of probiotic bacteria on liver functions in rats fed aflatoxins contaminated diet

Effect of probiotic bacteria on total protein, albumin, globulin and A/G ratio in rats fed aflatoxins contaminated diet

The results in Fig 3 indicated that the group received contaminated diet with aflatoxins showed high significant ($p<0.05$) decreased in total protein, albumin, globulin and A/G ratio. This agrees with those reported by Zohair (1996) in rats and Matri (2001) in Japanese quail birds.

The decreased in total protein, were improved by probiotic strains *Bifidobacterium bifidum* and *lactobacillus acidophilus* supplemented to aflatoxins treated group compared to aflatoxin group On the other hand albumin showed significant ($p\leq 0.05$) improvement indicating the capability of. probiotic bacteria to reduce the toxicity induced by aflatoxins.

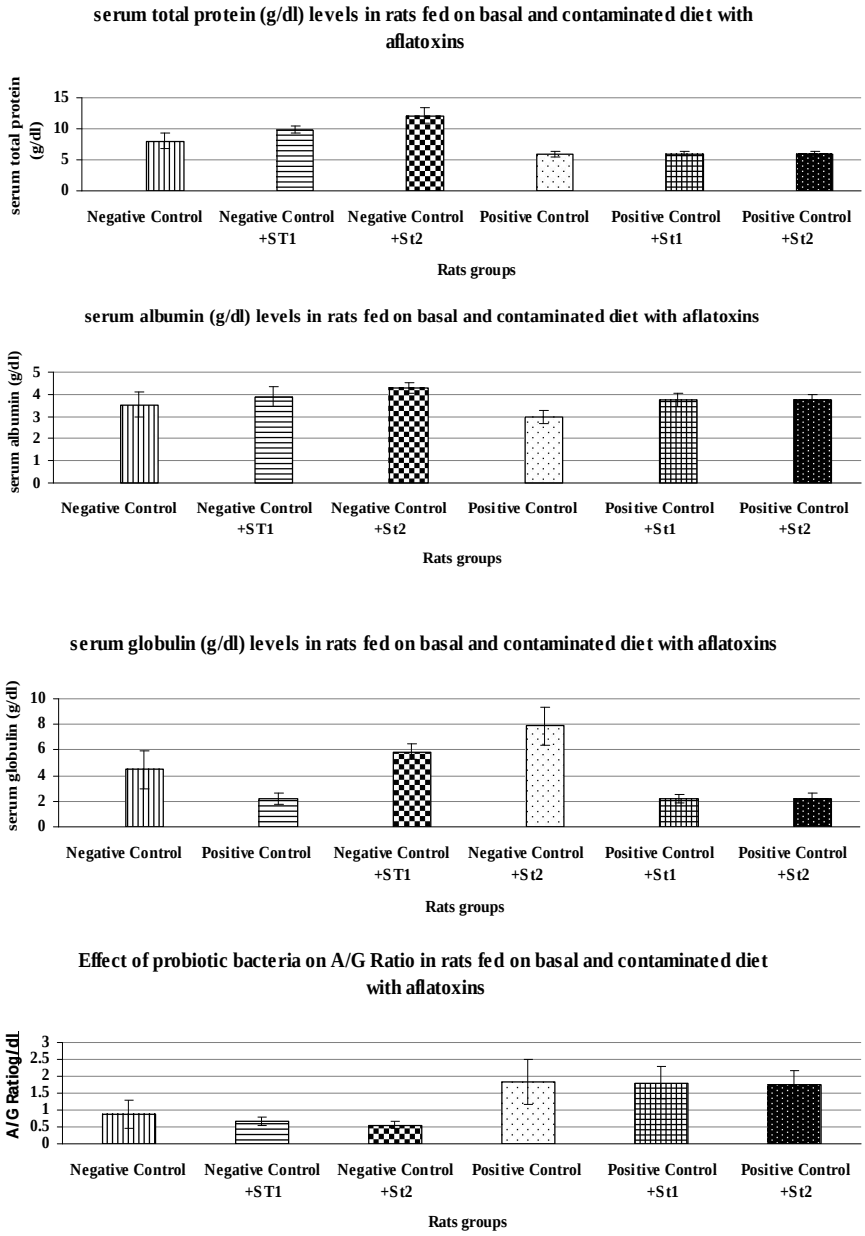

Fig.3:Effect of probiotic bacteria on total protein, albumin, globulin and A/G ratio in rats fed aflatoxins contaminated diet

Effect of probiotic bacteria on Kidney functions in rats fed aflatoxins contaminated diet

Fig 4 showed that The group received contaminated diet with aflatoxin showed very significantly ($p<0.05$) higher in urea and creatinine levels, as compared to healthy rats fed on basal diet indicated the toxicity of aflatoxin on kidney functions. These results were in coincide with those reported by Zohair (1996) in treated rats and those of Matri, (2001) in Japanese quail birds received contaminated feed with aflatoxin and showing significant higher ($p<0.05$) in serum total cholesterol, creatinine and urea. On the other hand the intakes of both probiotic bacteria strains significantly ($p\leq 0.05$) alleviated the elevation of urea level in aflatoxins treated rats. This result showed the detoxification activity of probiotic strains.

The probiotic with AFB1 bound to their surfaces likely to adhere to the intestinal wall and prolog exposure to dietary aflatoxin. Hence, specific probiotics may be potent and safe means to reduce absorption (Gratz *et al*., 2006). In addition the protective effects of probiotic bacteria against aflatoxin B1 induced intestinal and systemic toxicity via binding and reducing its transport in different tested systems (Gratz, 2007). The role of probiotic bacteria in improving the immunity may be also explained the detoxification activity of probiotic bacteria. There is now substantial evidence that probiotics can provide benefits by modulating immune functions. In animal models, probiotic supplementation is able to provide protection from spontaneous and chemically induced colitis by down regulating inflammatory cytokines or inducing regulatory mechanisms in a strain-specific manner (Borchers *et al.,* (2009).

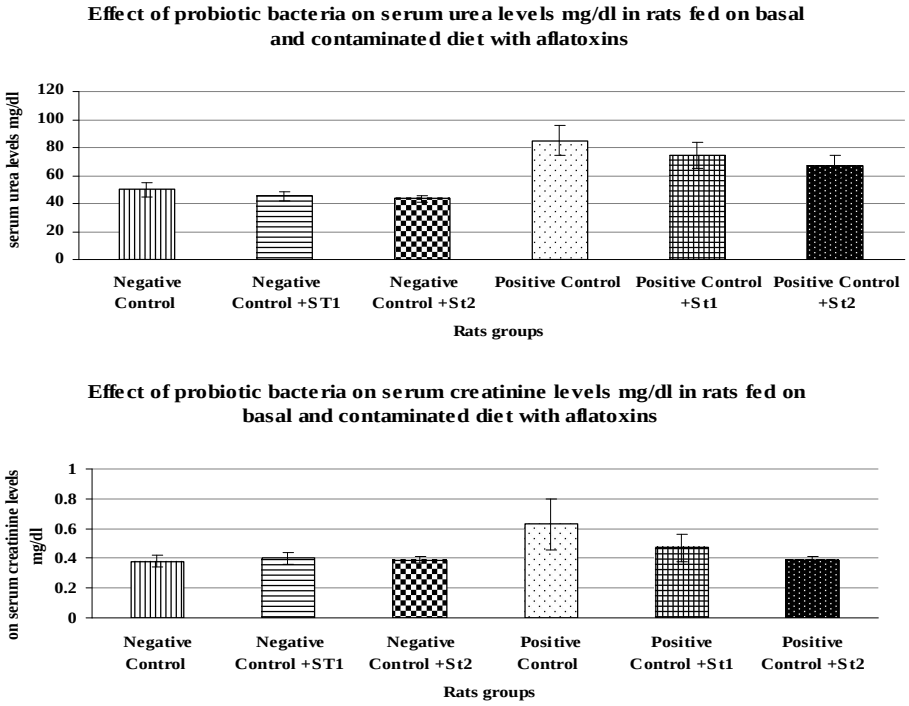

Fig 4: Effect of probiotic bacteria on total Kidney functions in rats fed aflatoxins contaminated diet

Effect of probiotic bacteria on organs weight of rats fed on aflatoxin contaminated diet

It could be noticed from Fig 5 that the group received contaminated diet with aflatoxins showed significantly ($p\leq 0.05$) increased in organs weight (heart, kidney) and very significant ($p<0.05$) increased in liver weight, as compared to basal diet group. The intake of probiotic bacteria showed significantly ($p\leq 0.05$) lower and improved organs weight in aflatoxins treated rats as compared to positive control. Gratz et al., (2006) suggested that by increasing the excretion of orally dosed aflatoxin via the fecal route, probiotic treatment prevents weight loss and reduces hepatotoxic effects caused by a high dose of AFB.

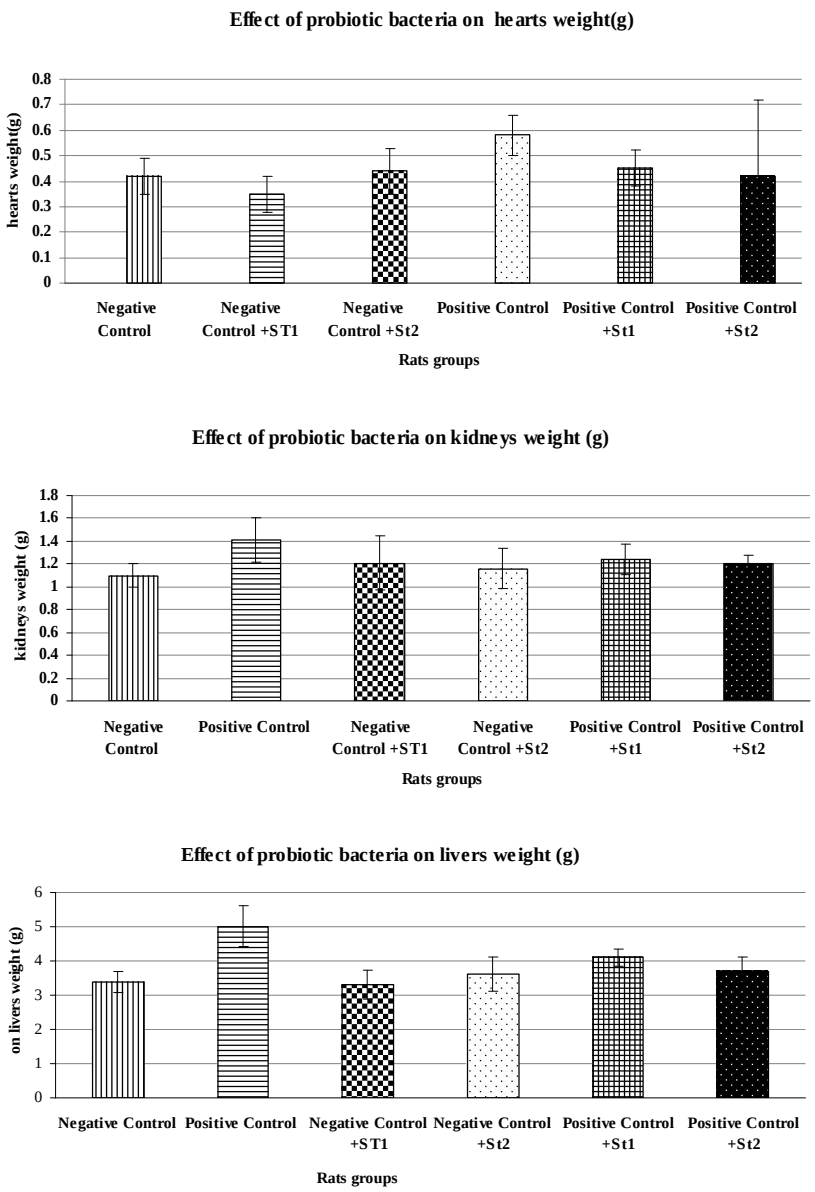

Fig. 5: Effect of probiotic bacteria on organs weight of rats fed on aflatoxin contaminated diet.

Results of histopathology

Kidneys of rat from group1 which was fed on basal diet for 6 weeks revealed the normal histological structure of renal parenchyma (photo 1). However, kidneys of rat from group 4 which was fed on contaminated diet with aflatoxin (10%/Kg diet) revealed marked dilatation and congestion of renal blood vessels and vacuolation of epithelial lining renal tubules (photo 2). Examined sections for groups 5: rats received aflatoxins + strain 1(*Bifidobacterium bifidum)* and group 6: aflatoxins +strain2 *(Lactobacillus acidophilus* showed no histopathological changes (photos 3, 4).

Liver of rat from group 1 which was fed on basal diet for 6 weeks revealed the normal histological structure of hepatic lobule (Photo 5). However, liver of rat from group 4 which was fed on contaminated diet with (10%/Kg diet) aflatoxin showed vacuolar degeneration of hepatocytes and fibrosis in the portal triad (Photo6). Some examined sections for group 5 which was fed on 10% contaminated diet with aflatoxin + strain 1*(Bifidobacteria)* showed no Histopathological changes except vacuolation of sporadic hepatocytes (Photo7), and other sections revealed no Histopathological changes (Photo8). Moreover, liver

of rat for group 6 which were fed on 10% contaminated diet with aflatoxin + strain 2*(L. acidophilus*) showed no Histopathological changes except dilatation and congestion of central vein and hepatic sinusoids (Photo9). Other sections from the same group revealed no Histopathological changes (Photo10).

The results of histopathology obtained indicate the toxicity of aflatoxins on liver and kidney, these results walk in the same line with numerous animal studies which have shown that the liver is the main target organ and therefore the main symptoms of aflatoxin exposure in domestic laboratory animals are hepatic injuries (Robins and Richard, 1992; IARC, 1993). In addition Matri, (2001) reported that sever histopathological changes was observed in the liver, kidney, heart, ovary and oviduct during aflatoxicosis. Also these results agree with Yener *et al*, (2009) who reported that the livers of the AF-treated group were slightly pale, enlarged and grayish mottled in appearance. However addition of probiotic strains to aflatoxin treated rats showed improved in the liver sections and showed no histopathological changes in kidneys as negative control. These results showed the effective role of probiotic bacteria especially the strains *Bifidobacteria* and *lactobacillus acidophilus* against toxicity induced by aflatoxins. These results are agree with Bekhatro, (2008) who reported that liver of rat fed on *B. bifidum* 29521 showed no histopathological changes except minute vacuoles in the cytoplasm of some hepatocytes.

In conclusion: the previous results indicated the protective action of probiotic strains *Bifidobacteria* and *lactobacillus acidophilus* as a potential protective agent against aflatoxin toxicity as well as their beneficial health effects and may thereby offer an effective dietary approach to decrease the risk cancer as a result of its ability of probiotic strains to bind with aflatoxins, inhibiting their absorption and protected against both memberane and DNA damage. Probiotics can also provide benefits by modulating immune functions. The data may be explained some of the antimutagenic and anticarcinogenic effects of probiotics microorganism.

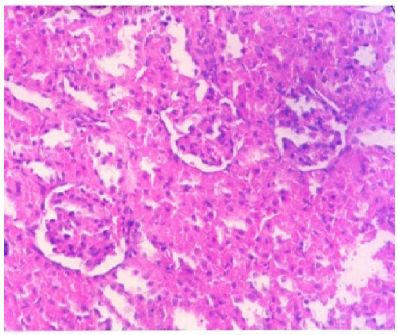

Photo (1): Kidney of rat from G1 showing the normal histological structure of renal parenchyma.

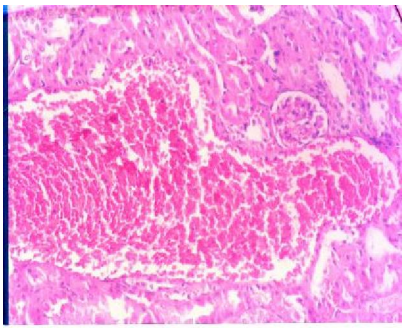

Photo (2): Kidney of rat from G4 showing marked dilatation and congestion of renal blood vessel.

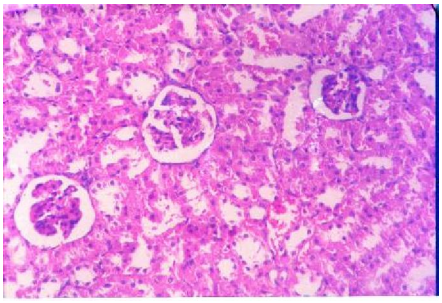

Photo (3): Kidney of rat from G5 showing no histopathological changes.

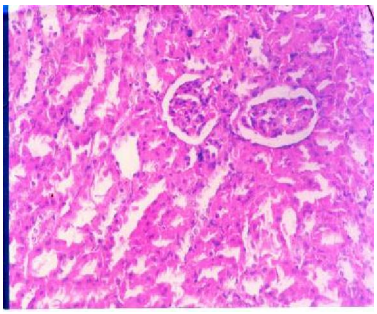

Photo (4): Kidney of rat from G6 showing no histopathological changes.

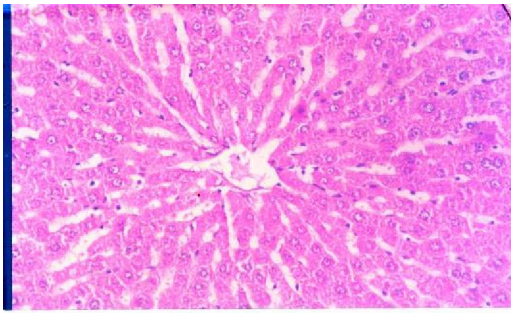

Photo (5): Liver of rat from G1 showing the normal histological structure of hepatic lobule.

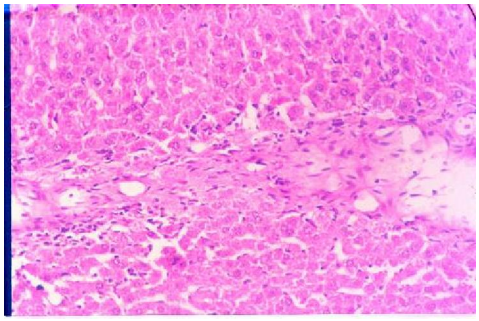

Photo (6): Liver of rat from G4 showing fibrosis in the portal triad.

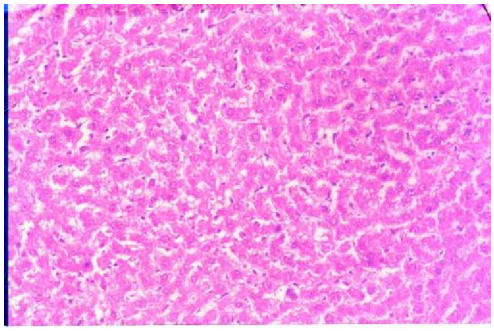

Photo (7): Liver of rat from G5 which showing no Histopathological except vacuolation of sporadic hepatocytes

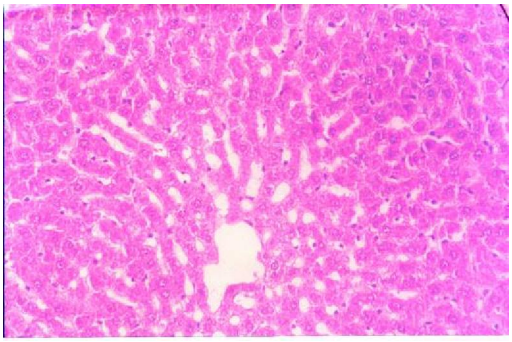

Photo (8): Liver of rat from G5 showing no Histopathological changes

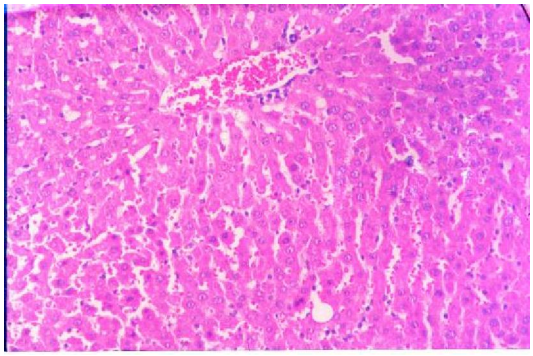

Photo (9): Liver of rat from G6 showed no histopathological changes except dilatation and congestion of central vein and hepatic sinusoids

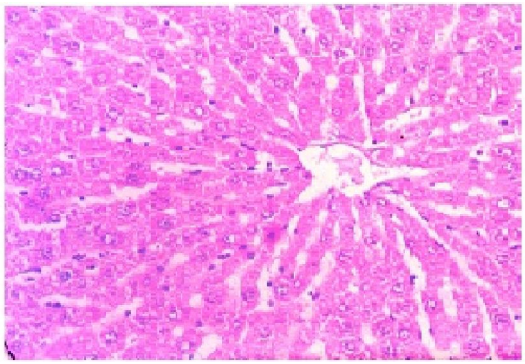

Photo (10): Other sections from liver of rat from G6 whichshowed no Histopathological Changes

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Study of the Right Liver Lobe Size /albumin Ratio as a Noninvasive Predictor of Oesophageal Varices Compared to: Spleen Size, Platelet count and Platelet count/spleen Diameter Ratio in Post Hepatitis C Virus Liver Cirrhosis in Egypt

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Abstract: Back ground and aim: Hepatic C Virus (HCV) is considered the most common aetiology of chronic liver disease in Egypt.Portal hypertension is a major complication of liver cirrhosis, and leads to the development of portosystmic shunts. Oesophageal varices are the most important among these shunts. Bleeding from oesophageal varices is the most serious complication of cirrhosis, with a high risk of death. The prevention of variceal bleeding is very important, non-selective beta blockers and prophylactic band ligation decrease the risk of bleeding by 50%. The current guide lines recommend screening of all cirrhotic patients by endoscopy, to identify patients at risk of bleeding so prophylactic treatment should be started to them. But repeated endoscopic examinations are unpleasant for patients, and carries high cost impact and more burden on endoscopic units, while only 50% of cirrhotic patients have esophageal varices, and up to 30% have large varices. For these reasons many non-invasive predictors for the presence and size of varices have been studied. The aim of this study to evaluate prospectively the right liver lobe size /albumin ratio and to compare it with spleen size, platelet count and platelet count/spleen diameter ratio as noninvasive predictors of oesophageal varices in post hepatitis C virus liver Cirrhosis in Egypt.Patients and methods:This prospective study included one hundred patients with post hepatitis C virus liver Cirrhosis. All studied subjects underwent a detailed history taking, clinical examination and a biochemical workup, including total bilirubin, aspartate aminotransferase, alanine aminotransferase, serum albumin, prothrombin activity, complete blood count and viral markers for hepatitis C and hepatitis B viruses. Child-Pugh score was calculated for all patients. An upper gastrointestinal endoscopy and abdominal ultrasound were performed for all patients. The platelet count to spleen diameter ratio and the right liver lobe to albumin ratio were calculated.Results: All the 4 predictors showed high statistically significant correlation with the presence and the grade of oesophageal varices (P values <0.001) Among the 4 noninvasive predictors the platelet count/spleen diameter ratio gave the highest accuracy at a cut-off value of 1326.58 (sensitivity 96.34% and specificity 83.33%) followed by the RT liver lobe/albumin concentration ratio at a cut-off value of 44.2 (sensitivity 91.46% and specificity 77.78%) followed by the spleen size at a cut-off value of 131.5mm(sensitivity 90.24% and specificity 83.33%) then lastly the platelet count at a cut-of value of 131000/mm^3(sensitivity 84.15% and specificity 83.33%).

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Key words: Noninvasive predictors of oesophageal varices, The right liver lobe/albumin ratio, The platelet count/spleen diameter ratio, Oesophageal varices, Post HCV liver cirrhosis.

1. Introduction:

Egypt has a very high prevalence of hepatitis C virus (HCV) and a high morbidity and mortality from chronic liver disease(1).HCV is considered the most common aetiology of chronic liver disease in Egypt, where prevalence of antibodies to HCV (anti-HCV) is 10-fold greater than in the United States and Europe (2).

Portal hypertension is a major complicationof liver cirrhosis, and leads to the development of portosystmic shunts. Oesophageal varices are the most important among these shunts due to its clinical effects and play a major role in transforming the disease from a pre-clinical to a clinical phase.Longitudinal studies have shown thatoesophageal and/or gastric varices eventually develop in all cirrhotic patients (3,4)and that once they have developed they tend to increase in size and to bleed (4). The yearly rate of development ofnew varices is about 5–10% (3,5); the rate of growth of varices from small to large ranges between 5% and 30% in different studies [5–8]. Bleeding from oesophageal varices is the most serious complication of cirrhosis, with a high risk of death [9].The mortality from each episode of variceal bleeding is17%-57% (4, 10, 11). On endoscopic examination the presence of red spots on the varices equals high risk of bleeding which is also related to the size of varices (12, 13).

The prevention of variceal bleeding is very important, non-selective beta blockers and prophylactic band ligation decrease the risk of bleeding by 50% (14,15). It is recommended that all cirrhotic patients should undergo endoscopic screening for the presence of varices (16–21), patients who has large or medium sized varices should be treated to prevent bleeding.

Patients who don't have varices and with compensated cirrhosis should repeat endoscopy every 2-3 years, andevery 1-2 years for those with small varices (17). It is also recommended for patients with decompensated cirrhosis to repeat endoscopy every 1 year even if there is no varices (17,19). But repeatedendoscopic examinations are unpleasant for patients, and carries high cost impact and more burden on endoscopic units, while only 50% of cirrhotic patients have esophageal varices, and up to 30% have large varices. For these reasons many non-invasive predictors for the presence and size of varices have been studied.

This study attempts to evaluate prospectively the right liver lobe size /albumin ratioand to compareit with spleen size, platelet count and platelet count/spleen diameter ratio as noninvasive predictors of oesophageal varices in post hepatitis C virus liver Cirrhosis in Egypt

2. Materials and methods:

This prospective study included one hundred patients with post hepatitis C virus liver Cirrhosis who were under investigations and treatment at the Gastroenterology & Hepatology outpatient clinics or those who were admitted to the Internal Medicine departments of the Cairo university hospitals.

Diagnosis of cirrhosis was based on physical findings, laboratory investigations and imaging findings. Patients who previously underwent injection sclerotherapy, band ligation, surgeryfor oesophageal varices, and those who were receiving beta blockers were excluded from the study. All patients with liver cirrhosis due to causes other than HCV were also excluded.

All studied subjects underwent a detailed history taking, clinical examination anda biochemical workup, including total bilirubin, aspartateaminotransferase, alanine aminotransferase, serum albumin, prothrombin activity, complete blood count and viral markers for hepatitis C and hepatitis B viruses. Child-Pugh score was calculated for allpatients using the 5 parameters (ascites, albumin, bilirubin, prothrombin activity and encephalopathy) (22). An upper gastrointestinal endoscopy and abdominal ultrasoundwere performed in all patients.

The right liver lobe diameter in the midclavicular line and the maximum spleen bipolar diameter were measured and the values were recorded. The platelet count to spleen diameter ratio and the right liver lobe to albumin ratio were calculated.

All endoscopies were performed in a single endoscopy unit by an experienced endoscopist and a grading classification I – IV was used (23). Grade I was used for varices in thelevel of mucosa, grade II for varices smaller than 5 mm filling less than 1/3 of the oesophageal lumen, grade III for varices larger than 5 mm filling more than 1/3 of the oesophageal lumen and grade IV for varices occupied more than 2/3 of esophageal lumen.

All the data were recorded, analyzed and correlated.

Data were statistically described in terms of range, mean ± standard deviation (± SD), median, frequencies (number of cases) and percentages when appropriate. Comparison of quantitative variables between the study groups was done using Mann Whitney *U* test for independent samples when comparing 2 groups and Kruskal Wallis analysis of variance (ANOVA) test with Mann Whitney *U* test for independent samples as posthoc multiple 2-group comparisons when comparing more than 2 groups. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. Accuracy was represented using the terms sensitivity, specificity, +ve predictive value, -ve predictive value, overall accuracy, the likelihood ratio of a positive test and the likelihood ratio of a negative test. Receiver operator characteristic (ROC) analysis was used to determine the optimum cut off value for the studied diagnostic markers. A probability value (*p* value) less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs Microsoft Excel 2007 (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

3. Results:

48 men and 52 women were included in the study, all with post HCV liver cirrhosis. The main clinical characteristicsof all patients are shown in table 1

The mean values of platelet (PLT) count, spleen diameter, PLT count/spleen diameter ratio and the right liver lobe diameter/albumin concentration ratio were correlated to the presence and grade of varices and they all highly significantly correlated to the presence and grade of varices as shown in table 2 and table 3.

The sensitivity, specificity and accuracy of PLT count, spleen diameter, PLT count/spleen

diameter ratio and the right liver lobediameter/albumin concentration ratio as noninvasive predictors of oesophageal varices were studied by applying the ROC curve to detect the cut off values with the best sensitivity and specificity.

Among the 4 noninvasive predictors the platelet count/spleen diameter ratio gave the highest accuracy at a cut-off value of 1326.58 followed by the RT liver lobe/albumin concentration ratio at a cut-off value of 44.2 followed by the spleen size at a cut-off value of 131.5mm then lastly the platelet count at a cut-of value of 131000/mm^3 as shown in table 4 and figures 1-5.

Table 1: showing the patients clinical characteristics

Main clinical characteristics of all patients	
Total number	100
Gender (M/F)	48/52
Age (mean ±SD)	49.23 ± 7.996
Age (range)	20 - 70
Child class.(A/B/C)	20/31/49
Varices present (yes/no)	82/18
Grade of varices(I/II/III/IV)	7/15/35/25
Varices type (small/large)	22/60
Mean PLT count,mm^3 (mean ±SD)	117070 ± 66145.883
Mean spleen diameter, mm (mean ±SD)	150.92 ± 23.371
Mean PLT count/spleen ratio(mean ±SD)	843.262 ± 616.250
Mean right liver lobe diameter, mm (mean ±SD)	147.74 ± 4.263
Mean albumin concentration,gm/dl (mean ±SD)	2.543 ± 0.9699
Mean right lobe/ albumin ratio (mean ±SD)	66.578 ± 23.735

Table 2: Correlation between all parameters with and without varices

Varices presence		PLT count	Spleen diameter	PLT/spleen ratio	Right lobe/albumin ratio
No	Mean	215,055.56	121.22	1,838.389	41.187
	SD	69,772.295	13.584	707.1507	8.8507
Yes	Mean	95,560.98	157.44	624.820	72.152
	SD	41,519.919	19.745	301.3943	22.3031
P		<0.001	<0.001	<0.001	<0.001

Table 3: Correlation between all predictors and grades of varices

Grade of varices		PLT count	Spleen diameter	PLT/spleen ratio	Right lobe/albumin ratio
I	Mean	167,428.57	136.29	1,204.285	42.192
	SD	59,969.040	15.966	364.5529	5.6384
II	Mean	99,466.67	149.87	668.750	56.054
	SD	37,015.183	16.296	246.9659	16.0342
III	Mean	96,000.00	160.60	600.033	72.150
	SD	31,167.479	14.136	199.6266	15.5372
IV	Mean	72,480.00	163.48	470.914	90.201
	SD	25,932.798	24.395	237.9270	20.3913
P		<0.001	0.007	<0.001	<0.001

Table 4: Comparison of accuracy of the 4 parameters in predicting the presence of oesophageal varices

Predictor	AUROC	Cut off point	Sensitivity (%)	Specificity (%)	(+)ve PV(%)	(-)ve PV(%)	Accuracy (%)	LR+	LR-
PLT count	0.912	131000	84.15	83.33	95.83	53.57	84.00	5.05	0.19
Spleen size	0.934	131.5	90.24	83.33	96.10	65.22	89.00	5.41	0.12
PLT count/spleen ratio	0.927	1326.58	96.34	83.33	96.34	83.33	94.00	5.78	0.04
Right liver lobe/Albumin conc.ratio	0.912	44.22	91.46	77.78	94.94	66.67	89.00	4.12	0.11

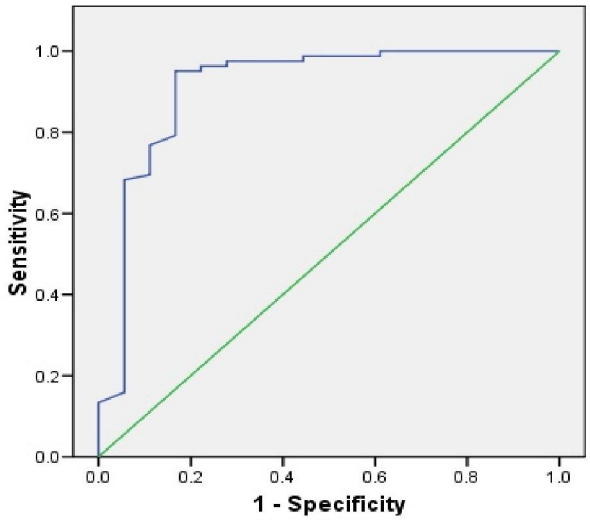

Figure1: ROC curve for sensitivity and specificity of platelet count for the prediction of varices

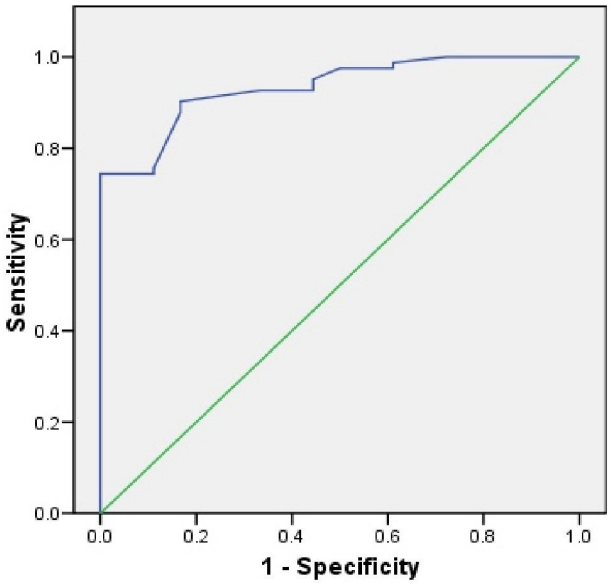

Figure 2: ROC curve for sensitivity and specificity of spleen size for the prediction of varices.

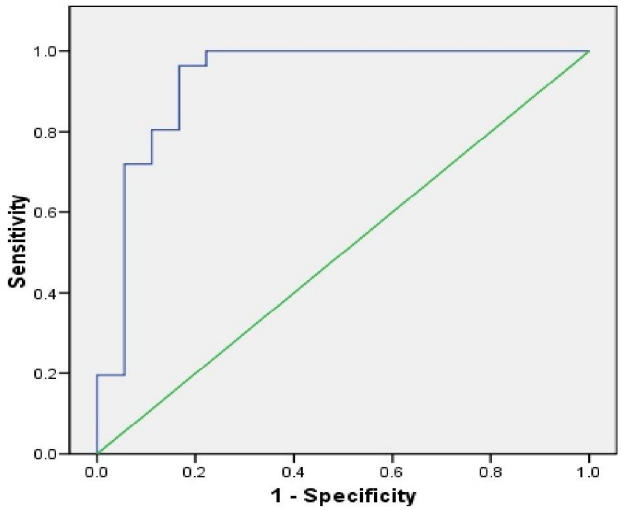

Figure 3: ROC curve for sensitivity and specificity of platelet count/spleen diameter ratio for the prediction of varices.

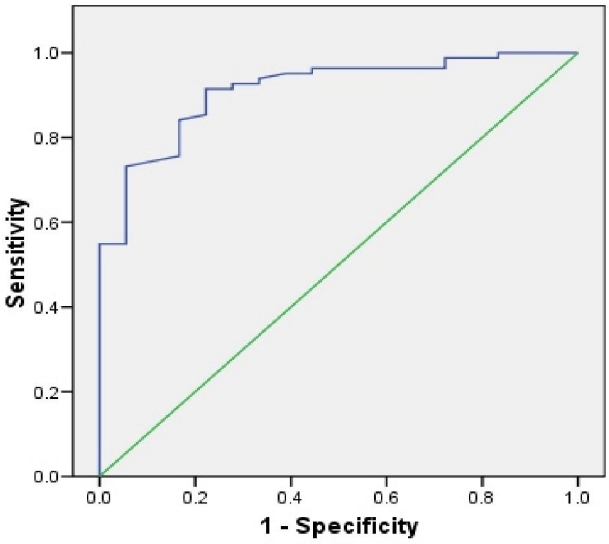

Figure 4: ROC curve for sensitivity and specificity of RT liver lobe size/albumin concentration ratio for the prediction of varices.

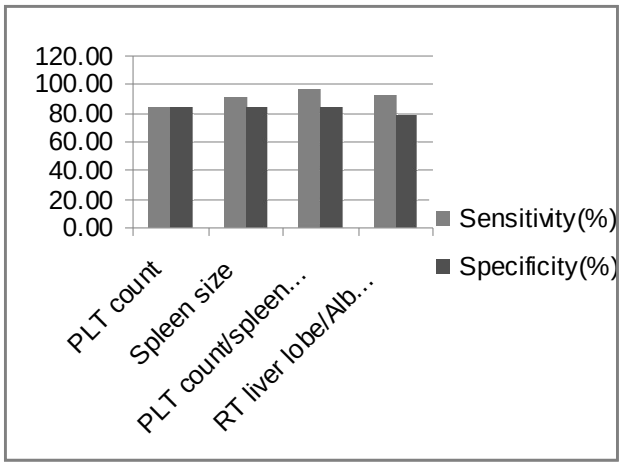

Figure 5: Comparison between sensitivity & specificity of the 4 parameters in predicting the presence of oesophageal varices

4. Discussion:

Bleeding oesophageal varices is still the leading cause of death in patients with cirrhosis. In recent studies, mortality rates vary between 11% and 20% within six weeks of the bleeding episode (24-27).

Endoscopy is still the gold standard method for diagnosis of oesophageal varices and is recommended every two to three years incirrhotic patients without varices, and every one to two years in patients with small varices (14,28,29).Several studies have been performed to find noninvasive parameters that can predict the presence of oesophageal varices in liver cirrhosis to reduce the cost andburden on endoscopy units(28).

The prevention of bleeding from oesophageal varices is an important goal. Identification of patients who are at risk of variceal bleeding is the first step in prevention of bleeding so the patients can be selected to start prophylactic treatment.

The prevalence of oesophageal varices among cirrhotics is variable, ranging from 24% to 80% (30). The value of diagnosing oesophageal varices by a noninvasive predictor is to save endoscopy to patients who have high probability of having varices.

In the present study as shown in tables 2-4 and figures 1 and 2 like many other previous studies (31-37) have shown that platelet count and spleen diameter correlate well with the presence of oesophageal varices.However, in cirrhoticpatients, the presence of thrombocytopenia may be due to several factors other than portal hypertension, as shortened mean platelet lifetime, decreased thrombopoietin production or myelotoxiceffects of hepatitis C viruse (38). The presence of splenomegaly in cirrhotic patients is mainly related to portal hypertension.

In 2003Giannini et al (28) introduced the use of the platelet count/spleen diameter ratio as a predictor of oesophageal varices. This ratio links thrombocytopenia to splenomegaly to introduce a variable that takes into consideration that thrombocytopenia is mainly due to hyperslenism secondary to portal hypertension.In his study with a cut-off value of 909 the sensitivity was 100% and specificity was 93%. In 2006 Giannini et al(39) reported the results of a multicenter study to validate the use of platelet count/spleen diameter ratio in the prediction of oesophageal varices. In this study the cut-off value of 909 showed sensitivity 92% and specificity 67%.Many studies (23, 39- 42) have been done using different best cut-off values to investigate thisparameter as a noninvasive predictor for oesophageal varices.

In the present study the cut-off value of 1326.58 for the platelet count /spleen diameter ratiowas used which showed sensitivity 96.34% and specificity 83.33% as shown in table 4 and figure 3.
In 2007 Alempijevic et al (24) investigated the right liver lobe diameter/albumin concentration ratio as a noninvasive predictor of oesophageal varices and at a cut-off value of 44.25 the sensitivity was 83.1% and the specificity was 73.9%. In the present study at a cut-off value of 44.22 for the right liver lobe diameter/albumin concentration ratio, the sensitivity was 91.46% and the specificity was 77.78% as shown in table 4 and figure 4.

5. Conclusion:

Among the noninvasive parameters studied in this study, the platelet count/spleen diameter ratio had the highest accuracy for diagnosing oesophagealvarices (sensitive to 96.34% and specificity 83.33%). For the right liver lobe diameter/albumin concentration ratio, the sensitivity was 91.46% and the specificity was 77.78% and can be considered as a noninvasive predictor of oesophageal varices that can provide accurate information as well as the platelet count/spleen diameter ratio.

The use of the 4 studied predictors in this study can help the physicians to restrict endoscopy on those who are highly suspected to have oesophageal varices to start the prophylactic therapy and not to use the endoscopy for all the patients.

Of course endoscopy still is the gold slandered for the diagnosis of oesophageal varices, but the use of the noninvasive predictors specially

platelet count/spleen diameter ratio and the right lobe liver size/albumin concentration ratio will be of a great help to reduce the number of endoscopies in patients with post hepatitis C virus liver cirrhosis in Egypt. More studies are required in a larger sample of post hepatitis C cirrhosis patients for validation of the right lobe liver size/albumin concentration ratio as a noninvasive predictor of oesophageal varices as well as the platelet count/spleen diameter ratio and to determine a cut-off value that can be safely recommended for the noninvasive diagnosis oesophageal varices.

The limitation of the present study includes: relatively small number of patients, liver biopsy was not done and the diagnosis of cirrhosis was based on clinical and laboratory results.

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Bitopological spaces via Double topological spaces

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Abstract: In this paper we shall study some bitopological properties via double topological spaces. We characterize the notions of pairwise continuous (resp. pairwise open, pairwise closed)(P .continuous, P - open, P - closed, for short) by a double continuous (resp. double open, double closed) mappings between double topological spaces. Also, we characterize the notions of P^* - continuous (resp. P^* -open, P^* - closed) by a supra double continuous (resp. open, closed) mappings between supra double topological spaces. Finally, we investigate the relationships between these types of mappings and give some counter examples.
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1. Introduction

The notion of a bitopological space (X , τ_1 , τ_2), that is a set X equipped with two topologies τ_1 and τ_2 was formulated by J. C. Kelly in [12].

There are several hundred works indicated to the investigation of bitopological spaces. The book [1] is a versatile introduction to the theory of bitopological spaces and its applications.

Flou set stems from some linguistic considerations of Yves Gentilhomme about the vocabulary of a natural language [6]. E. E. Kerre [13] introduced the mathematical definition of flou sets and binary operations on it.

In this paper we follow the suggestion of J. G. Garcia and S. E. Rodabaugh [5] that "double fuzzy set" is a more appropriate name 'than "intuitionistic fuzzy set", and therefore adopt the term "double-set" for the flou set, and "double-topology" for the flou topology.

There are several hundred works indicated to the investigation of double topology (eg [9, 10, 11, 15])

In this paper, making use the relation between bitopological spaces (BTS`s for short) and double topological spaces (DTS, for short), we characterize the notions of P - continuous (resp. P -open, P - closed) mappings by a double continuous (resp. open, closed) mappings.

Also, we introduce the notion of supra double topological space (SDTS, for short) and characterize the notion of P^* - continuous (resp, P^* -open, P^* - closed) mappings by supra double continuous (resp, open, closed) mappings.

Finally, we investigate the relationship between these types of mappings and give same counter examples.

Note that for the concepts and results that are used but not stated here we refer to [[2], [8], [14]].

1. Preliminaries:

In this section we shall present the fundamental definitions and concepts which will be needed in the sequel.

Definition 2.1.[9] i) A double set $\underline{A}$ (D- set for short) is an ordered pair

$(A_1, A_2) \in$ P (X) $\times$ P (X) such that $A_1 \subseteq A_2$.

ii) The family of all double sets on X, will be denoted by D(X), i.e.

$$D(X) = \{(A_1, A_2) \in P(X) \times P(X): A_1 \subseteq A_2\}.$$

iii) The double set $\underline{X}$ =(X, X) is called the universal double set, and $\underline{\varphi}$ = (φ , φ) is called the empty double set.

Definition 2.2. [9] Let $\underline{A}$ =(A_1 , A_2), $\underline{B}$ =(B_1 , B_2) $\in$ D (X). Then:

1) $\underline{A} \subseteq \underline{B} \Leftrightarrow A_i \subseteq B_i$, i =1,2

2) $\underline{A} = \underline{B} \Leftrightarrow A_i = B_i$, i =1, 2

3) $\underline{A} \cup \underline{B} = (A_1 \cup B_1, A_2 \cup B_2)$

4) $\underline{A} \cap \underline{B} = (A_1 \cap B_1, A_2 \cap B_2)$

5) $\underline{A}^C = (A_2^C, A_1^C)$ where $\underline{A}^C$ is the complement of $\underline{A}$.

6) Let $\eta_1, \eta_2 \subseteq P(X)$. The double product of η_1 and η_2 is denoted by $\eta_1 \hat{\times} \eta_2$ and is defined by $\eta_1 \hat{\times} \eta_2 = \{(A_1, A_2) \in \eta_1 \times \eta_2 : A_1 \subseteq A_2\}$.

Definition 2.3.[9] Let $f : X \to Y$ be a mapping, and let $\underline{A} \in D(X)$ and $\underline{B} \in D(Y)$.

The image of $\underline{A}$, defined by $f(\underline{A}) = (f(A_1), f(A_2))$ and the preimage $f^{-1}(\underline{B})$ is defined by $f^{-1}(\underline{B}) = (f^{-1}(B_1), [\quad], ([\quad]))$.

Definition 2.4 [9] Let X be a non- empty set. Then a family [\quad] [\quad] D (X) is called a double topology on X iff it satisfies the following axioms:

1) [\quad] = ([\quad], [\quad]), [\quad] = (X, X) $\in \eta$.

2) If $\underline{A}, \underline{B} \in \eta$, then $\underline{A} \cap \underline{B} \in \eta$.

3) If $\{\underline{A}_s : s \in S\} \subseteq \eta$, then $\bigcup_{s \in S} \underline{A}_s \in \eta$.

If η satisfies the axioms (1, 3), then it is called a supra double topology.

The pair (X, η) is called a double topological space. Each member of η is called an open double set in X. The complement of an open double set is called a closed double set. For any $\underline{A} \in D(X)$, the double closure of $\underline{A}$ is denoted by $\overline{\underline{A}}$ and is defined by $\overline{\underline{A}} = \cap \{\underline{B} : \underline{B} \in \eta^C \text{ and } \underline{A} \subseteq \underline{B}\}$.

Definition 2.5 [9] A mapping $f : (X, \eta) \to (Y, \theta)$ is called:

i) doubl continuous (D continuous for short) iff $f^{-1}(\underline{B}) \in \eta$ whenever $\underline{B} \in \theta$.

ii) doubl open (D open for short) iff $f(\underline{A}) \in \theta$ whenever $\underline{A} \in \eta$.

iii) doubl closed (D closed for short) iff $f(\underline{A}) \in \theta^C$ whenever $\underline{A} \in \eta^C$.

Remark: [11] Every DTS (X, η) define a BTS which is (X, π_1, π_2) where $\pi_1 = \{V_1 \subseteq X : \exists\, V_2 \subseteq X \text{ s.t } (V_1, V_2) \in \eta\}$ and $\pi_2 = \{V_2 \subseteq X : \exists\, V_1 \subseteq X \text{ S.T } (V_1, V_2) \in \eta\}$.

Conversely, every BTS (X, τ_1, τ_2) define a DT

$\tau_1 \hat{\times} \tau_2 = \{(A_1, A_2) \in D(X) : A_1 \in \tau_1, A_2 \in \tau_2\}$ on X associated with τ_1, τ_2.

Theorem 2.6.[9] If $f : (X, \eta) \to (Y, \eta^*)$ is a D-continuous function, then $f : (X, \pi_i) \to (Y, \pi^*_i)$ i = 1, 2 are continuous functions.

Theorem 2.7.[9] Let $(X, \tau_1 \hat{\times} \tau_2)$ be a DTS and (Y, η) be any DTS. Then

$f : (X, \tau_1 \hat{\times} \tau_2) \to (Y, \eta)$ is a D- continuous function iff

$f : (X, \tau_i) \to (Y, \pi_i)$ i = 1, 2 are continuous functions.

Definition 2.8.[3] A mapping $f : (X, \tau_1, \tau_2) \to (Y, \theta_1, \theta_2)$ is called P-continuous (resp, P -open, P-

closed) if f is τ_i - θ_i continuous(resp, open, closed), i=1, 2.

Definition 2.9. [4] A mapping I : P(X) $\longrightarrow$ P(X) is called supra- interior operator iff it satisfies the following axioms :

1) I (X) = X.
2) I (A) $\subseteq A$.
3) I (A I B) $\subseteq I$ (A) I I (B).
4) I (I (A)) = I (A).

Proposition 2.10. [4] Let (X, τ_1, τ_2) be a BTS. Then

i) $\tau^* = \tau_1 \bigsqcup \tau_2 = \{ U_1 \text{ Y } U_2 : U_i \in \tau_i \}$ is a supra topology on X and (X , τ^*) is the STS-associated to (X, τ_1, τ_2)

ii) The operator I : P(X) $\longrightarrow$ P(X), defined by I(A) = A^{01} Y A^{02}, A^{0i} is the τ_i - interior of A, (i=1,2), is a supra operator in which $\tau^* = \{ A \subseteq$ X: $A = I(A) \}$.

Proposition 2.11. [4] Let (X, τ_1, τ_2) be a BTS and (X , τ^*) its associated STS. Then C : P (X) $\longrightarrow$ P (X) defined by C (A)= $\overline{A}^1$ I $\overline{A}^2$.
is a supra- closure operator which induces the supra topology τ^*.

Remark 2.12 in [1], the author used the notion of pairwise open in a BTS, which means that: A is p-open $\Leftrightarrow A = U_1 \text{ Y } U_2$, $U_i \in \tau_i$ (i=1,2). In [4, 7], we used the same notion under the name of P^* - open or supra open in (X , τ^*), where τ^* is a supra topology generated by τ_1 and τ_2 . We say that $A \subseteq X$ is P^* - open (supra open) $\Leftrightarrow A \in \tau^*$.

Definition 2.13.[4]A mapping f : (X, τ_1, τ_2) $\longrightarrow$ (Y, θ_1, θ_2) is called

i) P^* -continuous iff f^{-1} (U) $\in \tau^*$ whenever U$\in \theta^*$.

ii) P^* -open iff f (V) $\in \theta^*$ whenever $V \in \tau^*$.

iii) P^* -closed iff f (V) $\in \theta^{*C}$ whenever $V \in \tau^{*C}$.

3. Operation on DTS and SDTS:

Definition 3.1. A mapping C : D(X) $\longrightarrow$ D(X) is a called double closure operator iff it satisfies the following axioms :

1) C ($\underline{\varphi}$) = $\underline{\varphi}$.
2) $\underline{A} \subseteq C$ ($\underline{A}$).
3) C ($\underline{A}$ Y $\underline{B}$) = C ($\underline{A}$) Y C ($\underline{B}$).
4) C (C ($\underline{A}$)) = C ($\underline{A}$).

If C satisfies the axioms (1, 2, 4) and the following axiom, it is called a supra double closure operator:

3*) C ($\underline{A}$ Y $\underline{B}$) $\supseteq C$ ($\underline{A}$) Y C ($\underline{B}$).

Theorem 3.2. Let (X, τ_1 , τ_2) be a BTS. The operator I_{12} : D(X) $\longrightarrow$ D(X), defined by I_{12} ($\underline{A}$)=([A_1 I A_2^{02}] ^{01}, A_2^{02}) $\forall$ $\underline{A} \in$ D (X), is a double interior operator which generates the double topology $\tau_1 \hat{\times} \tau_2$ on X.

Proof: As a sample, we prove the duality of the property ((3), definition 3.1) above, i.e. we prove that I_{12} ($\underline{A}$ I $\underline{B}$) = I_{12} ($\underline{A}$) I I_{12} ($\underline{B}$). The proof of the other parts are similar.

`` $I_{12}$ `` is a well defined map since $A_1 \subseteq A_2 \Rightarrow [A_1 \text{ I } A_2^{02}]^{01} \subseteq A_2^{02}$

3) I_{12} [$\underline{A}$ I $\underline{B}$]= I_{12} (A_1 I B_1, A_2 I B_2)= ([[A_1 I B_1] I [A_2 I B_2] ^{02}] ^{01},

[A_2 I B_2] ^{02})=(A_1^{01} I B_1^{01} I (A_2^{02}) ^{01} I (B_2^{02}) ^{01}, A_2^{02} I B_2^{02})

=([A_1^{01} I (A_2^{02}) ^{01}] I [B_1^{01} I (B_2^{02}) ^{01}], A_2^{02} I B_2^{02})

= ([A_1 I A_2^{02}] ^{01}, A_2^{02}) I ([B_1 I B_2^{02}] ^{01}, B_2^{02})
= I_{12} ($\underline{A}$) I I_{12} ($\underline{B}$)

Then I_{12} is a double interior operator and hence it generates a double topology η on X where

η ={ $\underline{A}$ | I_{12} ($\underline{A}$)= $\underline{A}$ }= { $\underline{A}$ | ([A_1 I A_2^{02}] ^{01}, A_2^{02})= $\underline{A}$ }

={ $\underline{A}$ |(A_1^{01} I A_2^{02}) ^{01}, A_2^{02}) =(A_1 , A_2)}=

{ $\underline{A}$ | $A_1^{01} = A_1 \wedge A_2^{02} = A_2$ }

={$\underline{A}$| $A_1 \in \tau_1 \wedge A_2 \in \tau_2$ }= $\tau_1 \hat{\times} \tau_2$.

Corollary 3.3. Let (X, τ_1 , τ_2) be any BTS. Then the operator

C_{12} : D (X) $\longrightarrow$ D (X) defined by: C_{12} ($\underline{A}$)=($\overline{A_1}^2$, $\overline{A_1^2 \text{ Y } A_2}^1$) $\forall$ $\underline{A} \in$ D (X) is a double closure

operator generates the double topology $\tau_1 \hat{\times} \tau_2$ on X.

Theorem 3.4. Let (X, τ_1, τ_2) be a BTS and let (X, τ^*) its associated supra topological space. Then the operator I^*: D (X) $\rightarrow$ D(X) defined by I^* ($\underline{A}$)= (I (A_1), I (A_2)),where I (A_i) = A_i^{01} Y A_i^{02} (i=1, 2), is a supra double interior operator such that $\tau_{I^*} = \tau^* \hat{\times} \tau^*$.

Proof: The proof that I^* is a supra-interior operator, follows from the definition of I^* and the fact that I is a supra –interior operator (prop. 2.9). For the proof of $\tau_{I^*} = \tau^* \hat{\times} \tau^*$, let $\underline{A}$ = (A_1 , A_2) $\in \tau^* \hat{\times} \tau^*$. Then $A_i \in \tau^*$, (i= 1, 2) and I (A_i) = A_i. SO, I^* ($\underline{A}$)=(I (A_1), I (A_2))= $\underline{A} \Rightarrow A \in \tau_{I^*} \Rightarrow \tau^* \hat{\times} \tau^* \subseteq \tau_{I^*}$.

Conversely, Let $\underline{A} \in \tau_{I^*}$. Then I^* ($\underline{A}$) = $\underline{A} \Rightarrow$ (I (A_1), I (A_2)) = (A_1 , A_2). Hence $A_i \in \tau^*$ (i= 1,2) and therefore $\underline{A}$ = (A_1 , A_2) $\in \tau^* \hat{\times} \tau^*$. So $\tau_{I^*} \subseteq \tau^* \hat{\times} \tau^*$ and consequently $\tau_{I^*} = \tau^* \hat{\times} \tau^*$.

Corollary 3.5. Let (X, τ_1, τ_2) be a BTS. Then the operator C^*: D (X) $\rightarrow$ D (X) such that C^* ($\underline{A}$) =($C(A_1)$, $C(A_2)$) ,where C (A_i)= $\overline{A}^1_i$ I $\overline{A}^2_i$ (i=1, 2) is a supra double closure operator such that $\tau_{C^*} = \tau^* \hat{\times} \tau^*$.

Theorem 3.6. Every double closure operator C : D (X) $\rightarrow$ D (X) generates a BTS (X, τ_1 , τ_2), where τ_i = {$A_i \subseteq$ X: C ((A_1 , A_2)^C) = (A_1 , A_2)^C , (A_1 , A_2)$\in$ D (X)}, i=1,2.

Proof: Straightforward.

4. The relations between P. continuous (resp P. open, P. closed) mappings and Duble continuous (resp duble open, double closed) mappings:

In this section, we characterize the notion of P-continuous (resp P -open, P -closed) by a D-continuous (resp D-open, D-closed) mappings.

Theorem 4.1. A mapping f : (X, τ_1 , τ_2) $\rightarrow$ (Y, θ_1, θ_2) is pairwise continuous iff f : (X, $\tau_1 \hat{\times} \tau_2$) $\rightarrow$ (Y, $\theta_1 \hat{\times} \theta_2$) is double continuous.

Proof: Let f : (X, τ_1 , τ_2) $\rightarrow$ (Y, θ_1, θ_2) be P-continuous and $\underline{B}$ = (B_1 , B_2) $\in \theta_1 \hat{\times} \theta_2$. Then f^{-1}(B_1)$\in \tau_1$ and f^{-1}(B_2)$\in t_2$. So, f^{-1} ($\underline{B}$) = (f^{-1} (B_1), f^{-1} (B_2))$\in \tau_1 \hat{\times} \tau_2$. Hence f : (X, $\tau_1 \hat{\times} \tau_2$) $\rightarrow$ (Y, $\theta_1 \hat{\times} \theta_2$) is double continuous.

Conversely: Let f : (X, $\tau_1 \hat{\times} \tau_2$) $\rightarrow$ (Y, $\theta_1 \hat{\times} \theta_2$) be D-continuous and let $G_1 \in \theta_1$. Then (G_1 , Y) $\in \theta_1 \hat{\times} \theta_2$. So, f^{-1} (G_1 , Y) = (f^{-1}(G_1), X) $\in \tau_1 \hat{\times} \tau_2$. Hence $f^{-1} G_1 \in \tau_1$ and f : (X, τ_1) $\rightarrow$ (Y, θ_1) is continuous function. Also, let $G_2 \in \theta_2$. Then (φ , G_2) $\in \theta_1 \hat{\times} \theta_2$. So f^{-1} (φ , G_2)=(φ , f^{-1} G_2)$\in \tau_1 \hat{\times} \tau_2$ .Hence f^{-1} $G_2 \in \tau_2$ and f : (X, τ_2) $\rightarrow$ (Y, θ_2) is continuous function. Therefore f : (X, τ_1 , τ_2) $\rightarrow$ (Y, θ_1, θ_2) is P - continuous.

Theorem 4.2. Let f : (X, τ_1 , τ_2) $\rightarrow$ (Y, θ_1, θ_2) be a mapping. Then the following conditions are equivalent:

1) f : (X, $\tau_1 \hat{\times} \tau_2$) $\rightarrow$ (Y, $\theta_1 \hat{\times} \theta_2$) is double continuous.

2) f^{-1}($\underline{B}$) $\in$ ($\tau_1 \hat{\times} \tau_2$)^C $\forall$ $\underline{B} \in$ ($\theta_1 \hat{\times} \theta_2$)^C

3) f (C_{12} ($\underline{A}$)) $\subseteq$ C_{12} (f ($\underline{A}$)) $\forall$ $\underline{A} \in$ D (X)

4) $C_{12}(f^{-1}(\underline{B})) \subseteq f^{-1}(C_{12}(\underline{B}))$ $\forall \underline{B} \in$ D (Y)

5) $f^{-1}(I_{12}\underline{B}) \subseteq I_{12} f^{-1}(\underline{B})$ $\forall \underline{B} \in$ D (Y)

Proof: (1) $\rightarrow$ (2): Let $\underline{B}=(B_1, B_2) \in (\theta_1 \hat{\times} \theta_2)^C$. Then $(B_1, B_2)^C \in \theta_1 \hat{\times} \theta_2 \Rightarrow$ $(B_2{}^C, B_1{}^C) \in \theta_1 \hat{\times} \theta_2 \Rightarrow f^{-1}(B_2{}^C, B_1{}^C) \in$ $\tau_1 \hat{\times} \tau_2 \Rightarrow$ $(f^{-1}(B_2{}^C), f^{-1}(B_1{}^C)) \in \tau_1 \hat{\times} \tau_2 \Rightarrow ([f^{-1}($ $B_2)]^C, [f^{-1}(B_1)]^C) \in \tau_1 \hat{\times} \tau_2 \Rightarrow (f^{-1}(B_1),$ $f^{-1}(B_2))^C \in \tau_1 \hat{\times} \tau_2 \Rightarrow (f^{-1}(B_1),$ $f^{-1}(B_2)) \in (\tau_1 \hat{\times} \tau_2)^C \Rightarrow$ $f^{-1}(\underline{B}) \in (\tau_1 \hat{\times} \tau_2)^C$.

(2) $\rightarrow$ (3): Let $\underline{A} \in$ D (X). Since $f(\underline{A}) \subseteq C_{12}(f(\underline{A}))$. Then $f^{-1} f(\underline{A}) \subseteq$ $f^{-1}[C_{12}(f(\underline{A}))] \Rightarrow \underline{A} \subseteq f^{-1}[C_{12}(f(\underline{A}))] \Rightarrow C_{12}(\underline{A})$ $\subseteq f^{-1}[C_{12}(f(\underline{A}))] \Rightarrow$ $f(C_{12}(\underline{A})) \subseteq C_{12}(f(\underline{A}))$(by (2))

(3) $\rightarrow$ (4): Let $\underline{B} \in$ D (Y). Take $\underline{A} = f^{-1}(\underline{B})$ using (3), we have $f[C_{12}(f^{-1}(\underline{B}))] \subseteq C_{12}(f f^{-1}$ $(\underline{B})) \subseteq C_{12}(\underline{B}) \Rightarrow$ $C_{12}(f^{-1}(\underline{B})) \subseteq f^{-1}(C_{12}(\underline{B}))$

(4) $\rightarrow$ (1): Let $G_1 \in \theta_1$. Then $(G_1, Y) \in \theta_1 \hat{\times}$ θ_2. Also, $(\varphi, G_1{}^C) \in$ $(\theta_1 \hat{\times} \theta_2)^C$, using (4) we have: $C_{12}(f^{-1}(\varphi, G_1{}^C)) \subseteq f^{-1}(C_{12}(\varphi, G_1{}^C))$ $= f^{-1}(\varphi, G_1{}^C) = (\varphi, f^{-1}(G_1{}^C)) \subseteq$ $C_{12}(\varphi, f^{-1}(G_1{}^C))$. So $f^{-1}(\varphi, G_1{}^C) \in (\tau_1 \hat{\times}$ $\tau_2)^C$. Hence $(f^{-1}(G_1), X) \in \tau_1 \hat{\times} \tau_2$. Therefore $f^{-1}(G_1) \in \tau_1$ and the mapping $f:(X, \tau_1) \rightarrow (Y, \theta_1)$ is continuous. Similarly we can show that $f:(X, \tau_2) \rightarrow (Y, \theta_2)$ is continuous. So $f:(X, \tau_1, \tau_2) \rightarrow (Y, \theta_1, \theta_2)$ is P- continuous. According to theorem 4.1, f is double continuous.

(1) $\rightarrow$ (5): Let f be double continuous. So, f : (X, τ_1, τ_2) $\rightarrow$ (Y, θ_1, θ_2) is P. continuous. Let $\underline{B} \in$ D (Y). Then $f^{-1}(I_{12}\underline{B}) =$ $f^{-1}((B_1 \text{ I } B_2{}^{02})^{01}, B_2{}^{02}) =$ $(f^{-1}(B_1 \text{ I } B_2{}^{02})^{01}, f^{-1} B_2{}^{02}) \subseteq ((f^{-1}(B_1 \text{ I }$ $B_2{}^{02}))^{01}, (f^{-1} B_2)^{02}) =$ $([f^{-1}(B_1) \text{ I } f^{-1}(B_2)^{02}]^{01}, (f^{-1} B_2)^{02}) =$ $I_{12} f^{-1}(\underline{B})$.

(5) $\rightarrow$ (1): Let $(B_1, Y) \in \theta_1 \hat{\times} \theta_2$ $\Rightarrow f^{-1}(I_{12}(B_1, Y)) \subseteq I_{12} f^{-1}((B_1, Y)) \Rightarrow$ $f^{-1}(B_1, Y) \subseteq I_{12} f^{-1}((B_1, Y))$ $\subseteq f^{-1}(B_1, Y)$. Then $f^{-1}(B_1, Y) =$ $I_{12} f^{-1}((B_1, Y))$ and therefore $f^{-1}(B_1, Y) \in$ $\tau_1 \hat{\times} \tau_2$. Hence $f^{-1} B_1 \in \tau_1$ and the mapping $f:(X, \tau_1) \rightarrow (Y, \theta_1)$ is continuous. Similarly if $B_2 \in \theta_2$, then $(\varphi, B_2) \in$ $\theta_1 \hat{\times} \theta_2$ and apply the condition, we have f^{-1} $(\varphi, B_2) \in \tau_1 \hat{\times} \tau_2$ Hence $f^{-1} B_2 \in \tau_2$ and the mapping $f:(X, \tau_2) \rightarrow (Y, \theta_2)$ is continuous. So, f : (X, τ_1, τ_2) $\rightarrow$ (Y, θ_1, θ_2) is P. continuous. So, according to theorem 4.1, f is double continuous.

By a similar way as in theorems 4.1, 4.2 we have the following theorems

Theorem 4.3. i) A mapping $f:(X,\tau_1,\tau_2)\to(Y,\theta_1,\theta_2)$ is P- open iff

$f:(X,\tau_1\hat{\times}\tau_2)\to(Y,\theta_1\hat{\times}\theta_2)$ is D open.

ii) A mapping $f:(X,\tau_1\hat{\times}\tau_2)\to(Y,\theta_1\hat{\times}\theta_2)$ is D- open iff

$f[I_{12}(\underline{H})]\subseteq I_{12}[f(\underline{H})],\forall\ \underline{H}\in D(X)$.

Theorem 4.4. A surjection mapping $f:(X,\tau_1\hat{\times}\tau_2)\to(Y,\theta_1\hat{\times}\theta_2)$ is D- open and D-continuous iff $f[I_{12}(\underline{H})]=I_{12}[f(\underline{H})],\ \forall\ \underline{H}\in D(X)$

Theorem 4.5. i) A mapping $f:(X,\tau_1,\tau_2)\to(Y,\theta_1,\theta_2)$ is P- closed iff

$f:(X,\tau_1\hat{\times}\tau_2)\to(Y,\theta_1\hat{\times}\theta_2)$ is D-closed.

ii) A mapping $f:(X,\tau_1\hat{\times}\tau_2)\to(Y,\theta_1\hat{\times}\theta_2)$ is D- closed iff

$C_{12}[f(\underline{H})]\subseteq f[C_{12}(\underline{H})],\forall\ \underline{H}\in D(X)$.

Theorem 4.6. A mapping $f:(X,\tau_1\hat{\times}\tau_2)\to(Y,\theta_1\hat{\times}\theta_2)$ is a D- closed and D-continuous iff $C_{12}[f(\underline{H})]=f[C_{12}(\underline{H})],\forall\ \underline{H}\in D(X)$.

Corollary 4.7. Let $(X,\tau_1\hat{\times}\tau_2)$ and $(Y,\theta_1\hat{\times}\theta_2)$ be double topological spaces. Then $f:(X,\tau_1\hat{\times}\tau_2)\to(Y,\theta_1\hat{\times}\theta_2)$ is a double homeomorphism iff

$f:(X,\tau_1,\tau_2)\to(Y,\theta_1,\theta_2)$ is P-homeomorphism.

5. The relations between P* -continuous (resp P* -open, P* -closed)mappings and supra double continuous (rep supra double open, supra double closed) mappings:

In this section, we shall study the relation between P* -continuous (resp P* -open ,P* - closed) mappings and supra- double topological spaces. The proofs of the following results are similar to the proof of the results in section 4. So, we prove theorem 5.2 as an example, and we shall omitte the proof of the others.

Theorem 5.1. Let (X,τ_1,τ_2) and (Y,θ_1,θ_2) be bitopological spaces. Also, let (X,τ^*) and (Y,θ^*) be their associated supra-topological spaces. The following equivalent:

1) $f:(X,\tau_1,\tau_2)\to(Y,\theta_1,\theta_2)$ is P* -continuous .

2) $f:(X,\tau^*\hat{\times}\tau^*)\to(Y,\theta^*\hat{\times}\theta^*)$ is SD-continuous.

3) $f^{-1}(\underline{B})\in(\tau^*\hat{\times}\tau^*)^C\ \forall\ \underline{B}\in(\theta^*\hat{\times}\theta^*)^C$

4) $f(C^*(\underline{A}))\subseteq C^*(f(\underline{A}))\ \forall\ \underline{A}\in D(X)$

5) $C^*[f^{-1}(\underline{B})]\subseteq f^{-1}(C^*(\underline{B}))\ \forall\ \underline{B}\in D(Y)$

6) $f^{-1}(I^*(\underline{B}))\subseteq I^*(f^{-1}(\underline{B}))\ \forall\ \underline{B}\in D(Y)$

Theorem 5.2. Let (X,τ_1,τ_2) and (Y,θ_1,θ_2) be bitopological spaces. Then the following are equivalent:

1) $f:(X,\tau_1,\tau_2)\to(Y,\theta_1,\theta_2)$ is P* open.

2) $f:(X,\tau^*\hat{\times}\tau^*)\to(Y,\theta^*\hat{\times}\theta^*)$ is a SD-open.

3) $f[I^*\underline{H}]\subseteq I^*[f(\underline{H})],\forall\ \underline{H}\in D(X)$.

Proof: $1\to2$ Let $\underline{A}=(A_1,A_2)\in\tau^*\hat{\times}\tau^*$. Then $f(A_i)\in\theta^*$ (i=1,2). So,

$f(\underline{A})=(f(A_1),f(A_2))\in\theta^*\hat{\times}\theta^*$. Hence $f:(X,\tau^*\hat{\times}\tau^*)\to(Y,\theta^*\hat{\times}\theta^*)$ is a SD-open function.

$2\to3$ Since, $I^*\underline{H}\subseteq\underline{H}\ \forall\ \underline{H}\in D(X)$. Then $f[I^*\underline{H}]\subseteq f(\underline{H})\Rightarrow I^*f[I^*\underline{H}]=f[I^*\underline{H}]\subseteq I^*f(\underline{H})$. Hence $f[I^*\underline{H}]\subseteq I^*[f(\underline{H})],\forall\ \underline{H}\in D(X)$.

$3\to1$ Straightforward.

Theorem 5.3. Let (X, τ_1, τ_2) and (Y, θ_1, θ_2) be bitopological spaces. A mapping $f:(X, \tau^* \hat{\times} \tau^*) \rightarrow (Y, \theta^* \hat{\times} \theta^*)$ is SD- open and SD-continuous iff

$f[I^*(\underline{H})] = I^*[f(\underline{H})]$ $\forall$ $\underline{H} \in$ D (X)

Theorem 5.4 Let (X, τ_1, τ_2) and (Y, θ_1, θ_2) be bitopological spaces. Then the following are equivalent:

i) $f:(X, \tau^* \hat{\times} \tau^*) \rightarrow (Y, \theta^* \hat{\times} \theta^*)$ is a SD-closed.

ii) f : (X, τ_1, τ_2) $\rightarrow$ (Y, θ_1, θ_2) is P* closed.

iii) $C^*(f(\underline{H})) \subseteq f[C^*(\underline{H})]$, $\forall$ $\underline{H} \in$ D(X)

Theorem 5.5. Let (X, τ_1, τ_2) and (Y, θ_1, θ_2) be bitopological spaces. A mapping

$f:(X, \tau^* \hat{\times} \tau^*) \rightarrow (Y, \theta^* \hat{\times} \theta^*)$ is SD- closed and SD- continuous iff

$C^*(f(\underline{H})) = f[C^*(\underline{H})]$, $\forall$ $\underline{H} \in$ D(X)

Corollary 5.6. Let (X, τ_1, τ_2) and (Y, θ_1, θ_2) be bitopological spaces. Then

$f:(X, \tau^* \hat{\times} \tau^*) \rightarrow (Y, \theta^* \hat{\times} \theta^*)$ is a SD-homeomorphism iff

f : (X, τ_1, τ_2) $\rightarrow$ (Y, θ_1, θ_2) is P* - homeomorphism function

6. Relation between D continuous (open, closed) mappings and SD continuous (open, closed) mappings:

Theorem 6.1. Let (X, τ_1, τ_2) and (Y, θ_1, θ_2) be bitopological spaces and let,

f : (X, τ_1, τ_2) $\rightarrow$ (Y, θ_1, θ_2) be P- continuous (resp P- open). Then

$f:(X, \tau^* \hat{\times} \tau^*) \rightarrow (Y, \theta^* \hat{\times} \theta^*)$ is SD-continuous (resp SD-open).

Proof: It follows from the definition of P-continuous (resp P- open) and SD-continuous (resp SD-open).

Theorem 6.2. Let (X, τ_1, τ_2) and (Y, θ_1, θ_2) be bitopological spaces and let

f : (X, τ_1, τ_2) $\rightarrow$ (Y, θ_1, θ_2) be P- closed and injection. Then

$f:(X, \tau^* \hat{\times} \tau^*) \rightarrow (Y, \theta^* \hat{\times} \theta^*)$ is SD-closed.

Proof: Let f : (X, τ_1, τ_2) $\rightarrow$ (Y, θ_1, θ_2) be a P- closed and injection. Let $\underline{H} \in \tau^{*C} \hat{\times} \tau^{*C}$. Then $\underline{H} = (K_1 \cap K_2, G_1 \cap G_2)$ such that $K_i, G_i \in \tau_i^C$ (i=1, 2). Then

$f(\underline{H}) = f(K_1 \cap K_2, G_1 \cap G_2) = (f(K_1 \cap K_2), f(G_1 \cap G_2)) \subseteq (f(K_1) \cap f(K_2), f(G_1) \cap f(G_2))$. $f(K_i), f(G_i) \in \theta_i^C \Rightarrow$

$f(\underline{H}) \in \theta^{*C} \hat{\times} \theta^{*C}$. Hence $f:(X, \tau^* \hat{\times} \tau^*) \rightarrow (Y, \theta^* \hat{\times} \theta^*)$ is SD-closed.

Note that the mapping may be SD-continuous (SD-open and SD-closed) but not double continuous (double open and double closed) mapping as shown in the following example:

Example 6.3. Let X={a, b, c}. τ_1={ φ, X, {a}, {b, c}}, τ_2={ φ, X, {c}, {a, b}}. Also, let Y= {p, q, r} θ_1={ φ, Y, {r}, {p, q}}, θ_2={ φ, Y, {p}, {q, r}}. Then

$\tau_1 \hat{\times} \tau_2$ = { $\underline{\varphi}$, $\underline{X}$, (φ,{c}), (φ, {a, b}), (φ, X), ({a}, {a, b}), ({a}, X), ({b, c}, X)}

$\theta_1 \hat{\times} \theta_2$ = { $\underline{\varphi}$, $\underline{Y}$, (φ,{p}), (φ, {q, r}), (φ, Y), ({r}, {q, r}) , ({r}, Y), ({p, q}, Y)}

let f : (X, τ_1, τ_2) $\rightarrow$ (Y, θ_1, θ_2) such that f (a) = p, f (b)= q, f (c)=r. Then f is not D-open since (φ ,{c}) $\in$ $\tau_1 \hat{\times} \tau_2$ $\Rightarrow$ f (φ ,{c}) = (φ,{r}) $\notin$ $\theta_1 \hat{\times} \theta_2$

let τ^* = { φ, X, {a}, {b, c}, {c}, {a, b},{a, c}}. Then τ^* is not a topology since {b, c} $\cap$ {a, b}= {b} $\notin$ τ^*. Also,

θ^* = { φ, Y, {r}, {p, q}, {p}, {q, r}, {r, p}} θ^* is not a topology since {p, q} $\cap$ {q, r}= {q} $\notin$ θ^*. Then

$\tau^* \hat{\times} \tau^*$ ={ $\underline{\varphi}$, (φ, {a}), (φ, {b, c}),(φ, {c}), (φ, {a, b}), (φ, {a, c}), (φ,X), ({a}, {a}), ({a}, {a, b}),

({a}, {a, c}), ({a}, X), ({c}, {c}), ({c}, {b, c}), ({c}, {a, c}), ({c}, X), ({b, c}, {b, c}), ({b, c}, X), ({a, b}, {a, b}), ({a, b}, X), ({a, c}, {a, c}), ({a, c}, X), $\underline{X}$} and

$\theta^* \hat{\times} \theta^*$ ={$\underline{\varphi}$, (φ, {r}), (φ, {p}), (φ, {p, q}), (φ, {q, r}), (φ, {r, p}), (φ, Y), ({r}, {r}), ({r}, {r, q}), ({r}, {r, p}), ({r}, Y), ({p}, {p}), ({p}, {p, q}), ({p}, {r, p}), ({p}, Y), ({p, q}, {p, q}), ({p, q}, Y), ({q, r}, {q, r}), ({q, r}, Y), ({r, p}, {r, p}), ({r, p}, Y), $\underline{Y}$}.

Let $f:(X, \tau^* \hat{\times} \tau^*) \to$(Y, $\theta^* \hat{\times} \theta^*$). Then f is SD-continuous but

f : (X, $\tau_1 \hat{\times} \tau_2$) $\to$ (Y, $\theta_1 \hat{\times} \theta_2$) is not D-continuous, since

(φ ,{p}) $\in$ $\theta_1 \hat{\times} \theta_2$ but f^{-1} (φ ,{p}) = (φ,{a})$\notin$ $\tau_1 \hat{\times} \tau_2$.

Let $f:(X, \tau^* \hat{\times} \tau^*) \to$(Y, $\theta^* \hat{\times} \theta^*$). Then f is SD-open but

f : (X, $\tau_1 \hat{\times} \tau_2$) $\to$ (Y, $\theta_1 \hat{\times} \theta_2$) is not D- open, since

(φ ,{c}) $\in$ $\tau_1 \hat{\times} \tau_2$ but f (φ ,{c}) = (φ,{r})$\notin$ $\theta_1 \hat{\times} \theta_2$. Finally,

Let $f:(X, \tau^* \hat{\times} \tau^*) \to$(Y, $\theta^* \hat{\times} \theta^*$). Then f is SD-closed but

f : (X, $\tau_1 \hat{\times} \tau_2$) $\to$ (Y, $\theta_1 \hat{\times} \theta_2$) is not D-closed, since

({a, b}, X)$\in$ $(\tau_1 \hat{\times} \tau_2)^C$ but f ({a, b}, X) = ({p, q}, Y)$\notin$ $(\theta_1 \hat{\times} \theta_2)^C$.

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Synthesis and some applications of Anionic Palmitic Acid Schiff Base Salt Surfactants

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Abstract: Schiff bases derived from condensation reaction of benzaldehyde or anizaldehyde and diethylenetriamine were prepared. The products were reacted with palmatic acid (1 : 1 *mol) to give the corresponding palmitic Schiff base salt surfactants . The chemical* structures of the prepared compounds were confirmed using elemental analysis, FTIR and ^{1}H-NMR spectroscopy. Various surface properties of the synthesized surfactants were evaluated particularly, critical micelle concentration, effectiveness, efficiency, maximum surface excess and minimum surface area . These surfactants were also evaluated as corrosion inhibitors and as biocide agents Gram positive and Gram negative bacterial strains. The rheological properties, and the filter loss for oil-based mud (invert - emulsion mud) were evaluated, the result showed that they were a good emulsifiers and filter loss control agent for oil – base mud. It has been found that they have good corrosion inhabitation for low carbon steel alloy and has good bactericidal effect.

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Key words: Surfactants, Corrosion inhibitors, oil base mud and biological activity.

1. Introduction:

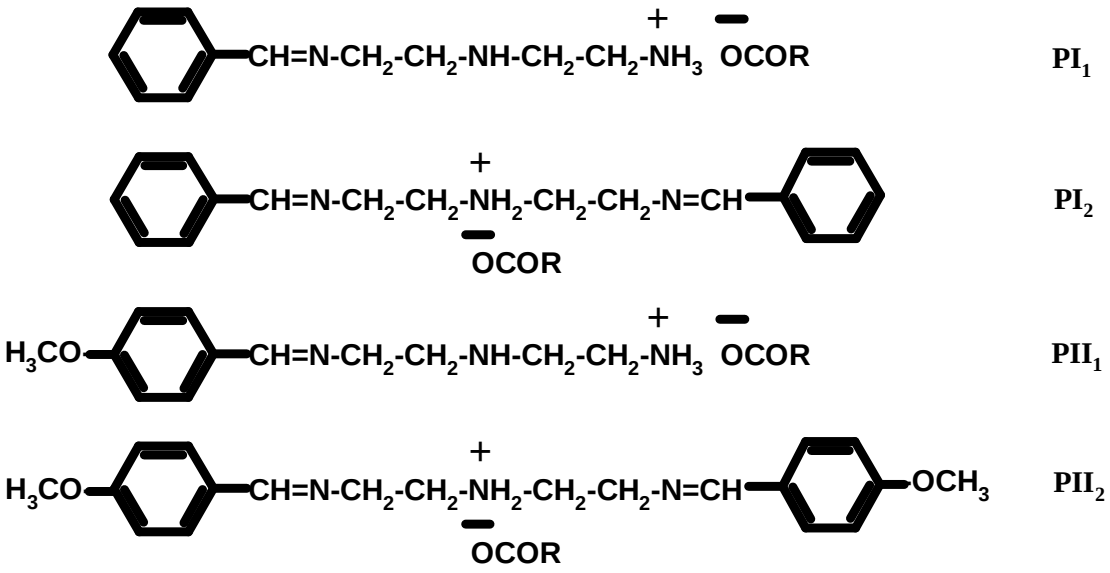
Schiff base compounds are the condensation product of an amine and a ketone/aldehyde. Recent publications showed increased attention to these compounds as corrosion inhibitors especially inacidic environments for various metals like steel, aluminium and copper [1-7]. The greatest advantage of many Schiff base compounds is that they can be conveniently and easily synthesized from relatively cheap materials. The inhibition of steel corrosion by acids has been previously studied by various researchers using different organic compounds [8–12]. These compounds in general are adsorbed on the metal surface blocking the active corrosion sites. Several Schiff bases have been investigated as corrosion inhibitors for various metals and alloys in acidic media [1, 2, and 13].

Schiff bases are characterized by the –N=CH– (imine) group which is important in elucidating the mechanism of transamination and racemisation reactions in biological systems [14&15]. Due to the great flexibility and diverse structural aspects, a wide range of Schiff bases have been synthesized and their complexation behavior studied [16]. On the other hand, in the rotary drilling there are a variety of functions and characteristics that are expected from drilling fluids (drilling mud or simply mud). The drilling fluid is expected to carry cuttings from beneath the bit, transport them up the annulus and permit their separation at the surface while at the same time the rotary bit is cooled and cleaned . A drilling fluid is also intended to reduce friction between the drill string and the sides of the hole while maintaining, preventing corrosion fatigue of the drilling– pipe and allowing interpretation of electric logs. There are a various advantage of using oil – based drilling mud in rotary drilling , In summary, wells drilled with oil- based mud normally produce lower waste volumes than those drilled with water based mud , Also the penetration of the formation by water is avoided , thus preventing swelling or sloughing . One of the most important properties of these drilling fluids are their thermal stability and that they don't present rheological and thixtropic problems under the condition of drilling [17-20]. The use of Schiff base as an emulsifier in oil-base mud is a novel. The function of the emulsifier in oil-based mud is to impart weak gel strength and also emulsification of additional water which is picked up during the drilling operation that promotes a stable emulsion [21-22].

2. Material and Experimental Techniques:

Preparation of the surfactant compounds

Benzaldehyde, (1 or 2 mol) or anizaldehyde, (1 or 2 mol) was condensated with diethylenetriamine in ethanol forming the corresponding Schiff base, each products was reacted in water with palmitic acid (1:1 mol) forming palmitic acidamine salts surfactants (PI_1, PI_2, PII_1 and PII_2) having the following structures :



Where R = $C_{15}H_{31}$

Fig. 1: The chemical structure of the prepared compounds

Surface Tension Measurements:

Surface tension measurements were made for freshly prepared surfactant solutions with concentration range from ($1x\ 10^{-1}$ to $1x\ 10^{-5}$) mol/L. The test was done at 25°C using Du Nouy Kruss-K6. The surfactants solutions were prepared in 1M HCl solutions. The surface tensions were the average of three readings for the each sample.

Emulsification power:

Emulsification power of the synthesized surfactants was measured by vigorous shaking of 10 ml surfactant solution (0.1%) and 10 ml paraffin oil for 5 minutes at 25 °C. The emulsification power was expressed as the time required for separation of 9 ml of pure water.

Corrosion Measurements:

Weight loss Technique:

A weight-loss technique [ASTM G31-72 (Reapproved 2004)] was used to measure the inhibiting efficiency to corrosion of the prepared Schiff bases amphiphiles for mild steel in 1MHCl solutions at 25°C for 24h. The dissolved oxygen range is 6-8 ppm. The experiments were performed with mild steel specimens having a composition (wt %): 0.17 C, 0.035 Si, 0.51 Mn, 0.82 P, and the remainder is Fe. Each specimen was machined into regular shapes of 55.8-cm^2 cross-sectional area. The specimens were sequentially abraded with different emery papers, degreased with acetone, washed with distilled water and dried. Corrosive solutions is 1M HCl in the absence and presence of the inhibitors at concentrations ranging from to 2.7 to $270X10^{-6}$M were prepared from doubly distilled water . The average of three coupons was recorded.

Polarization for corrosion (Tafel):

Electrochemical tests have been evaluated by using a Voltalab -40 Potentiostat PGZ 301 at 25°C. HCl1 (1M) was used as corrosive solution in the absence and presence of the inhibitor. The concentrations ranging from 1 to $30X10^{-6}$M of compound PII_2 were prepared using doubly distilled water. The efficiency was determined from the following equation

$$\text{Eff. \%} = (CR_{Bl} - CR_{inh})/CR_{Bl}$$

Where: CR_{Bl} is the corrosion rate in absence of inhibitor and CR_{inh} is the corrosion rate in presence of inhibitor

Tests for oil – base mud:

The materials and chemical additives of oil – base mud were obtained from the Baroid Company to be used as a reference sample (R). The work with oil – base emulsion mud or oil – water ratio (70/30) were performed by using newly prepared surfactants (PI_1, PI_2, PII_1 and PII_2) as primary emulsifiers with ratio (2%) of the mud formulation and compared to oil – base mud formulated with imported primary emulsifier (R) (commercial one)[23].

Mud formulation:

Mud Formulation were as follow : diesel oil (350 ml + primary emulsifier 2% (10 ml) + tap water (150 ml) were mixed for 20 minutes and then (1.99%) viscosifier + secondary emulsifier (6 ml) + organphilic clay (1.5%) + soda lime (1.59%) for all

muds formulation , All chemical additives were added slowly using stirring and mixed well in the mixer. So we have:

MR: Mud formulation of oil- base ratio (70/30) with the imported (commercial emulsifier) (R).

MPI_1: Mud formulation of oil- base ratio (70/30) with the new prepared emulsifier PI_1.

MPI_2: Mud formulation of oil- base ratio (70/30) with the new prepared emulsifier PI_2.

$MPII_1$: Mud formulation of oil- base ratio (70/30)with the new prepared emulsifier PII_1.

$MPII_2$: Mud formulation of oil- base ratio (70/30) with the new prepared emulsifier PII_2.

All the samples were aged to 300 ^oF for 16 hours and tested at 75 ^oF , The tests were conducted on both aged and unaged samples.

Biological activity:

The synthesized surfactants were screened for their biocidal activity using diffusion disc method. A filter paper sterilized disc saturated with measured quantity of samples (20 mg in 1 ml DMSO) is placed on plate containing solid bacterial medium (nutrient agar broth) or fungal medium (Dox's medium) which has been heavily seeded with the spore suspension of the tested organism. After inoculation, the diameter of the clear zone of inhibition surrounding the sample is taken as inhibitory power of samples against the particular test organism.

3. Results and Discussion:

3.1. Structure:

The chemical structure of the synthesized Schiff – based surfactants (alkyl amine salts) were confirmed using micro elemental analyses, which showed good coincidence between the calculated and found values of C, H, and N (%) Table1.

FTIR spectra showed the following bands; ν_{NH} at 3453 and 3296 cm^{-1}, $\nu_{C=O}$ at 1736 cm^{-1}, $\nu_{C=N}$ at 1640cm^{-1}, ν_{C-H} at 2844 cm^{-1} and ν_{N^+} at 3179 cm^{-1} which confirmed the expected functional groups found in the synthesized molcules.

^{1}HNMR analyses of compounds (PI_1, PI_2, $PII_1$and PII_2) as representative samples showed the following; for PI_1 : δ =1.29 ppm (H, C-H), δ=0.96 ppm (S, 3H of terminal CH_3),δ=7.29-8.29 ppm (5H-benzylidene),δ= 2.77 ppm (t, 2H, CH_2), δ=2.23ppm (t,2H,CH2-COO), and δ= 2.0 ppm (2H, NH_2 proton). The data of elemental analysis, FTIR and ^{1}HNMR spectra confirms the chemical structures of the synthesized amphiphiles as represented in Fig1.

Table (1): Characterization of prepared Schiff base amine salt surfactants

Compound	Yield%	C %		H %		N %	
		Calc.	Found	Calc.	Found	Calc.	Found
PI_1	70	72.48	72.40	10.96	10.76	9.39	9.18
PI_2	85	75.26	75.75	9.9	9.2	7.85	8.18
PII_1	80	70.44	69.97	10.69	9.95	8.80	8.18
PII_2	90	72.6	72.48	9.4	9.2	7.05	6.84

3.2. Surface Properties:

Surface tension (γ) and critical micelle concentration, (CMC)

Figure (2) represent the variation of surface tension (γ) against –log (conc.) of the synthesized surfactants (PI_1, PI_2, PII_1 and PII_2) at 25˚C in acid solutions.

It is clear that the surface tension curve have two characteristic regions. One at the lower concentrations, which showed gradual decrease in the surface tension while the other at higher concentrations at which the surface tension stays almost constant with very small changes. The higher change in the first region indicates the fast adsorption of the surfactant molcules towards the interface. At the higher concentrations, the surface is almost saturated by the surfactant molcules and no further molcules penetrate to the interface, hence, the change in the surface tension will be very small.

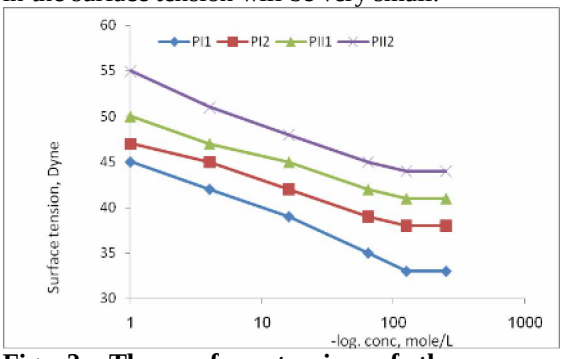


Fig. 2: The surface tension of the prepared compounds.

The intercept between these two regions indicate the critical micelle concentration (CMC) of each surfactant. Table (2) represents the critical micelle concentration (CMC) values of the synthesized Schiff base surfactants. The values of CMC in Table 2 revealed that the presence of the hydrophobic chain length of the synthesized surfactants produces a decrease in their values. That behavior could be explained as the number of methylene groups in the surfactant molcules increases their hydrophobicity which increases the hydrophobic chain solvent interaction. Hence, the molcules tend to form aggregates in the bulk of their solutions. As we can see the presence of the terminal methoxy group in compounds (PII_1 and PII_2) increases their hydrophilicity, which was responsible for decreasing their CMC values and increasing the depression of the surface tension at this concentration, (Table 2).

From table (2) the highest CMC value was observed for PII_1 $24x10^{-5}$ at 25°C while, the lowest value was observed for PII_2 $13x10^{-5}$ at 25°C which referred to the difference in their structures where, the presence of the oxygen atom in the skeleton of PII decreases the water / hydrophobe repulsion that occurred through hydrogen bond formation [24].

Effectiveness (π_{cmc}), maximum surface excess (Γ_{max}) and minimum surface area (A_{min})

The difference between surface tension values of the surfactant solution at its CMC and that of the corresponding distilled water is defined as effectiveness (π_{cmc}). The most efficient is the one that gives the greatest lowering in surface tension at (CMC). According to the result of effectiveness shown in Table2, PII_1 is a mostly found to be more efficient, It achieves the maximum reduction of the surface tension at CMC.

The maximum surface excess is expressed as the concentration of surfactant molcules at the interface per unit area (Γ_{max}).While the minimum surface area is defined as the area occupied by each molcule in nm^2 at the interface. Using the adsorption law of molcules at the interfaces, (Γ_{max}) values were calculated according to the following equation.

$$\Gamma_{max} = \left(\frac{d\gamma}{d \log c}\right) / (8.3x10^{7} x\ RT)$$

Where d γ/dlog C is the surface pressure, R, universal gas constant and T, the absolute temperature. Regarding the results listed in Table 2 we observe that Γ_{max} decreases by increasing the molar ratio of the Schiff based structure for both PI_2 and PII_2 which may be attributed to desolation of molcules from the interface and their dissolution in the aqueous phase [25].

The minimum surface area occupied by each surfactant molcules at the air\water interface (A_{min}) is calculated according to the following equation.

$$A_{min} = 1/N.\ \Gamma_{max}$$

where (A_{min}) values increase with the increase in molar ratio of Schiff base for both PI_2 and PII_2

Standard free energies of micellization and adsorption (ΔG^{o}_{mic}, ΔG^{o}_{ads})

According to the Gibb's equations of thermodynamics, the thermodynamic functions of micellization and adsorption were calculated from the surface parameters as listed in (Tables 2) according to the Gibb's equations of thermodynamics as follows:

$$\Delta G_{mic} = -2.303RT\log (CMC)$$

$$\Delta G_{ads} = \Delta G_{mic} - (0.6023\ X\ 10^{-1}\ X\ \pi_{cmc}\ X\ A_{min})$$

From Table (2) the standard free energy change of adsorption (ΔG°_{ads}) was found to be more negative than that for the micellization process (ΔG°_{mic}), which refers to the higher tendency of these surfactants to adsorb at air/water interface rather than the micellization. The preference of adsorption is governed by the thermodynamic stability of the molcules at the air/water interface. Thus rising in ΔG°_{ads} can be traced to the presence of the methoxy group within the surfactant molcules, which increase the adsorption process at the air/water interface. The hydrogen bonds occurring between the water and surfactant molcules provide good stability for the adsorbed molcules at the interface. These results are in good agreement with the data obtained from A_{min} values [26].

Table (2): Surface properties of prepared Schiff base amine salt surfactants

Comp.	CMC,mol E-5	γ_{cmc}	Πcmc	Γ_{max}E-10	$A_{min\ nm}^{2}$	$-\Delta G_{mic}$	$-\Delta G_{ads}$
PI_1	19	41.8	30.2	0.96	1.73	21.14	24.28
PI_2	20	45.0	27.0	0.90	1.84	21.07	24.07
PII_1	24	33.6	38.4	1.13	1.46	20.69	24.08
PII_2	13	38.4	33.6	1.02	1.63	22.24	25.54

3.3. Emulsion Stability:

The emulsifying power values of the prepared compounds are listed in table (3). As shown in the table (3) it is clear that all prepared surfactants have high emulsion stability. it is clear also that the PII_1 or PII_2 compounds have higher emulsifying power values than that of PI1or PI2 . This is due to the solubility of the attached counter ions. It is well known that the emulsion stability of the surfactant molcule depends mainly on its hydrophob part, which increases, as the carbon chain length increases the emulsion stability of the compound. In our study the hydrophob part in the prepared compounds is constant. Consequently, the emulsion stability depends on the counter ions attached with the prepared compounds. The solubilities of the counter ions of PII_1 and PII_2 are more than that of $PI_1$and PI_2 due to the presence of ether group.

The emulsion stability of PI_1 is more than that of PI_2. This is due to the solubility of counter ion of the compound PI_1 which is more than that of PI2 due to the presence of imine and benzyl groups. For the same reason, the emulsifying power of the PII_1 is more than that of PII_2.

Table (3): Emulsion stability of 0.1% surfactants in 10% NaCl water

Comp.	PI_1	PI_2	PII_1	PII_2
Time, Minute.	34	22	37	33

3.4. Corrosion Inhibition:

The inhibition efficiencies of the synthesized Schiff base amphiphiles were calculated by weight loss before and after immersion in the corrosive medium according to the equation:

$$\eta\ (\%) = [(W_o - W)/ W_o] \times 100$$

Where W_o and W are the corrosion weight losses of the uninhibited and inhibited steels respectively.

Table 4 and Figures 3 represent the variation of inhibition efficiencies of the synthesized inhibitors in different acidic media for mild steel on wide range of doses (from 2.7 up to $270X\ 10^{-6}M$). It is clear that the inhibition efficiency towards corrosion process increases by increasing the inhibitor dose. The maximum corrosion inhibition was found at $30X10^{-6}M$ for all the synthesized inhibitors in HCl solutions. Increasing the inhibition efficiencies with increasing the concentration of the synthesized inhibitors is mainly due to the adsorption of those inhibitors on the metal surface. The adsorption mechanism of the inhibitor molcules at metal/solution interfaces is depending on the chemical structure of the inhibitors and their response towards the environment governed by one or more of the following topics:

1. Electrostatic attraction between the charged inhibitor molcules and the metal surface.
2. Interaction between the p-electrons in the inhibitor molcules and the metal.
3. Interaction between uncharged moieties in the inhibitor molcules and the metal surface.

The chemical structures of the synthesized inhibitors comprise unsaturation sites (conjugation within benzene rings) and heteroatom in the imino groups. The conjugation in the benzenoid nucleus of benzaldehyde and anisaldehyde interacts with the metal surface forming a strong adsorption bonds. This interaction also occurrs due to the imino group of the Schiff bases.

The hydrophobic part of the palmetic acid acts as the uncharged moiety which forms the thin film preventing chloride ions from the metal surfaces. The proposed mechanism of the steel dissolution in the acidic medium was described in the following equations [27]:

$$Fe + Cl^- \rightarrow (FeCl^-)_{ads}$$

$$(FeCl^-)_{ads} \rightarrow (FeCl)_{ads} + e^-$$

$$(FeCl)_{ads} \rightarrow FeCl^+ + e^-$$

$$FeCl^+ \rightarrow Fe^{+2} + Cl^-$$

$$Fe + H^+ \rightarrow (FeH^+)_{ads}$$

$$(FeH^+)_{ads} + e^- \rightarrow (FeH)_{ads}$$

$$(FeH)_{ads} + H^+ + e^- \rightarrow Fe + H_2$$

Meanwhile, imino groups are protonated in the acidic medium forming the protonated imine ($-N^+H=C-$) which is adsorbed physically to the negative species formed during steel dissolution ($FeCl^-$). In case of the synthesized inhibitors containing anisaldehyde moiety, the methoxy group containing uncharged electron pairs on the oxygen atoms. The uncharged electron pair interacts with the positively charged species produced during the steel dissolution in the acidic medium (FeH^+).

The experimental results of the corrosion processes of the mild steel in the acidic media showed high inhibition efficiencies of the synthesized inhibitors. The inhibition efficiencies of the synthesized inhibitors are 88%, 35%, 46% and 68% at lower concentrations ($2.7X10^{-6}M$) for PI_1, PI_2, PII_1 and PII_2, respectively. The maximum inhibitions (at higher concentration of $270X10^{-6}M$) are 98%, 98.4%. 97.7%, and 99%, for PI_1, PI_2, PII_1 and PII_2, respectively,

Analyzing the data of corrosion inhibition reveals that the inhibitors derived from two imino groups exhibit higher efficiencies than those obtained from one derivative. This could be refereed to the combination of the three above mentioned topics in their inhibition mechanism. However, two Schiff base molcules derivatives are stronger than one molecule. The above results was confirmed by the thermodynamic parameters of the micellization and adsorption process where it is found that the more negative ΔG^{o}_{mic} and ΔG^{o}_{ads} are the more corrosion efficiency increases.

Table (4): The corrosion inhibition efficiency of the prepared Schiff base amine salt surfactans at different doses (weight loss method).

Conc.M*10^6	Eff., %			
	PI_1	PI_2	PII_1	PII_2
2.7	88	35	46	68
5	90	67	77	86
10	93	87	86	93
30	96	97	96	97
65	96	97	97	98
130	97	98.2	97	98.3
270	98	98.4	97.7	99

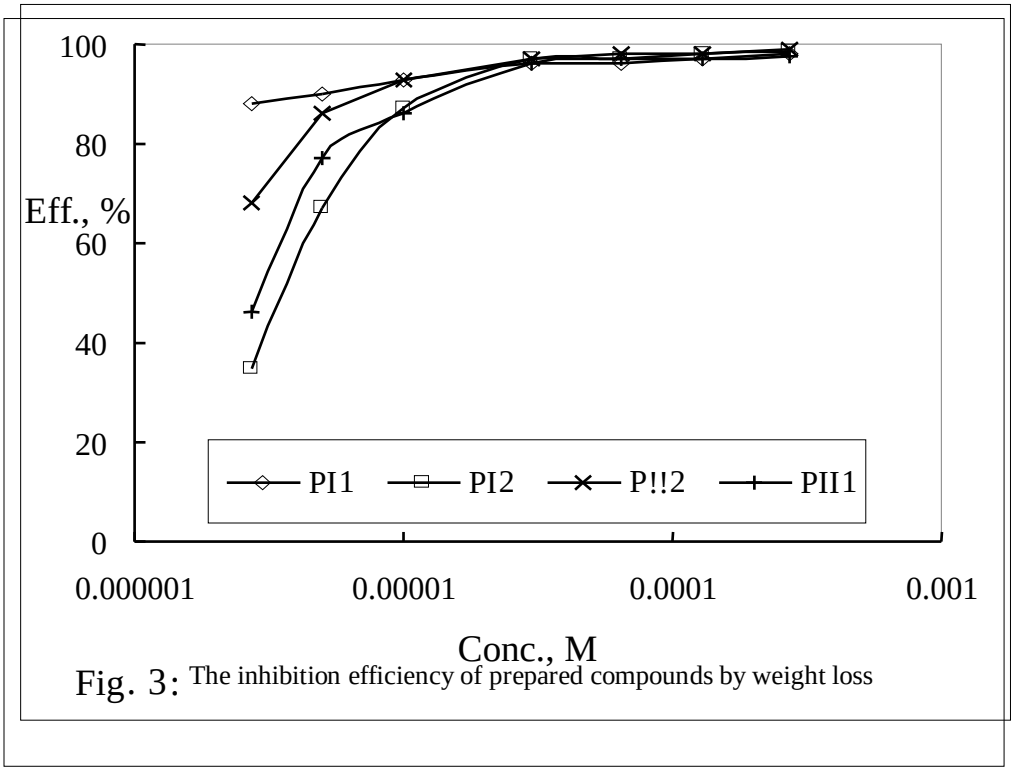


Fig. 3: The inhibition efficiency of prepared compounds by weight loss

Tafel polarization

Table 5 shows a typical record of Tafel polarization measurements for mild steel in 1M HCl in the absence and presence of the PII_2. Corrosion current density (i_{corr}) of bare mild steel electrode in this condition was 3769 Acm^{-2}. It is clear that corrosion current density decreased with increasing the concentration of the PII_2.

It's clear that addition of the PII_2 to acid media affected both the cathodic and anodic parts of the curves. Therefore, these compounds behave as mixed inhibitors and Corrosion potential is shifted to the positive direction more markedly. This shows that the effect of inhibitors on the anodic reaction is more observable than on the cathodic reaction. Increasing the concentration of the PII_2 caused corrosion potential to be nobler, although there was no specific relation between E_{corr} and inhibitors concentration.

Table (5): Tafel polarization parameter values for the corrosion of mild steel in 1M hydrolic acid containing different concentrations of the PII_2.

Conc ($MX10^{-6}$)	-E Corr, mv	I. corr, mA/cm^2	Rp $ohm.cm^2$	Ba, mv	Bc, mv	Corr.rat, mmy	EFF., %
0.0	516	0.3769	220.65	155.7	-168.0	4.41	0.0
1	534.3	0.1939	392.35	221.1	-146.9	2.26	48.75
2	547.5	0.1086	497	261	-127.3	1.269	71.12
3	552.4	0.0999	486.7	224.9	-125.7	1.168	73.5
6	544.4	0.0806	553.68	266.8	-122.4	0.942	78.64
10						0.552	87.48
30						0.146	96.69

Table 5 shows the polarization parameters for corrosion of mild steel in the presence of different concentrations of the investigated compound. The corrosion inhibition efficiency increases when the concentration of the inhibitor increases. Depolarization of both anodic and cathodic branches after addition of the inhibitors indicated no dissolution of metal and the maximum efficiency was 96, 69% for concentration of $30X10^{-6}$M PII_2.

3.5. Oil - Base mud

The prepared Schiff-base surfactants (PI_1, PI_2, PII_1, and PII2) were evaluated as a primary emulsifier for oil-base mud with the oil-water ration (70/30) and emulsifier concentration 2%. The evaluation incorporates the study of rheological properties (apparent viscosity (AV), plastic viscosity (pv) yield point (yp), gel strength G_{10} second , G_{10} mint, Thixtropy and the filter loss of the mud are formulated by the new prepared emulsifiers compared to the reference mud sample (MR). The test was carried under the condition of high temperature 350°F and high pressure 500 psi with continuous circulation for 16 hours. Table (6) shows the results of the test before and after aged for both the mud formulated with the new emulsifiers and a reference sample mud.

Table (6) : Rheological properties (apparent viscosity AV, plastic viscosity PV , yield point YP , Gel strength and filter loss) after and before aged for 16 hours at 350 ° F and 500 (psi) .

Mud example	Temperature F^O	Rheological properties			Gel strength		Filter loss (ml)
		A.V (CP)	P.V (CP)	Y.P ($1b/100ft^2$)	G / 10 sec	G/10 mint	
M_R	Initial	17	15	16	6	6	9
	300 ° F	13	11	5	2	3	12
MPI_1	Initial	20	17	16	9	9	7
	300 ° F	17	13	8	3	4	9
MPI_2	Initial	18	15	12	7	7	7,5
	300 ° F	15	12	7	4	5	8
$MPII_1$	Initial	22	14	17	8	8	6
	300 ° F	18	16	11	4	5	10
$MPII_2$	Initial	19	16	12	6	6	6
	300 ° F	15	11	6	2	1	9

Rheological properties:-

Before ageds:- The apparent viscosity were 20, 18, 22, 19 (cp) for Mpl_1, MpI_2, $MpII_1$, and $MpII_2$ respectively while the (AV) for MR was 18 (cp), plastic viscosity (PV) were 17, 15, 14, 16 for MPI_1, MPI_2, $MP11_1$, $MPII_2$ while the pv for MR was 15 (cp), The yield point were 16, 12, 17, 12 16/100ft2 for MPI, MpI_2, $MpII_1$, $MpII_2$ respectively while the yield point for MR was 16 $1b/100Ft^2$.

Gel strength G10 second recorded 9,7,8,6 $1b/100Ft^2$ and G_{10} mint showed were also 9,7,8, $61b/100Ft^2$ for the four oil-base mud formulated with the new emulsifiers . So the Thixtropy for each one is equal to zero which is compatible with Thixtropy of reference sample MR which is also equal zero.

Filter loss of the mud formulated with the new emulsifier MPI, MP_2 $MPII_1$, $MpII_2$ were equal to 7, 7.5, 6, 6ml while the filter loss of the reference sample was 9 ml

The decrease of filter loss of the mud formulated with the new emulsifiers and the reference sample mud indicates the stability of the mud.

After aged:-

The apparent viscosity (Av) and plastic viscosity (pv) yield point. (yp) decreased as a result of increasing temperature up to 350°F. For the 4 mud formulation with the new emulsifiers MpI_1, MpI_2, $MpII_1$, $MpII_2$ the apparent viscosities were (17, 15, 18, 15) cp while the apparent viscosity of the reference mud sample MR was 11 cp. Also plastic viscosities were 13cp for MpI_1, 12 cp for MPI_2, 16 cp for $MPII_1$ and $MpII_2$ where the plastic viscosity of MR was II cp after aged. The yield point was 8, 7, 11 and 6 $lb/100Ft^2$ for 4 new emulsifier mud formulations where the yield point for MR was 5 $1b/100ft^2$. This result indicates that mud formulation with new emulsifiers was better than or compatible with the reference mud sample. Filter loss of the mud formulations with the new emulsifiers were less than filter loss of reference mud sample (MR) after aged [28].

From the above results we can conclude that the Rheological properties, gel strength and filter loss of the mud formulated with the new Schiff base emulsifiers showed results which were better than the reference sample mud, and that the Schiff base surfactants were good emulsifiers for oil-base mud. They are in order PI_1> PII_1>PII_2>PI_2. The best emulsifier is PI_1.

3. 6. Biocidal Activity

The biological biocidall activity of the synthesized surfactants amine salts against Gram-negative bacteria (Eschericha coli) and (Neisseria gonorrhea) Gram-positive bacteria (Staphylococcus aureus) and (Streptococcus faecalis) is represented in Table 7. The biocidal activity of the compounds under investigation (PI_1, PI_2, PII_1 and PII_2) is due to adsorbance at the water/cell membrane interface. This adsorption increases solubility through the cell membrane increasing its permeability towards the media ingredients and correspondingly. Thus biological reactions disturb within the cell cytoplasm. Table 7 shows that, the synthesized surfactants have a good biocidel activity against bacteria used in this investigation. Table 7 shows that, compounds PI_1 and PI_2 give relatively better inhibition zones against bacteria than compounds PII_1 and PII_2.

The mechanism of action of such surfactants on bacteria is understood to be one of electrostatic interaction and physical disruption, as opposed to interference with a metabolic pathway, as is commonly the situation with antibiotic species [29]. After the cationic site of the agent attached to a significant lipophilic component binds to anionic sites of the cell wall surface it is then able to diffuse through the cell wall and bind to the membrane. Acting as a surfactant, it is able to disrupt the membrane and permit the release of electrolytes and nucleic materials, leading to cell death. The membrane activity of the surfactants depends on the character of the polar head groups (size and electric charge distribution) and hydrocarbon chains (length, saturation and multiple chains).

Table (7): Biocidal activity for the prepared Schiff base surfactants expressed by Inhibition zone diameter (mm/mg)

Sample	Eschericha coli (G-)	Neisseria gonorrhea (G-)	Staphylococcus albus (G+)	Streptococcus faecalis (G+)
Control	0.0	0.0	0.0	0.0
PI_1	14	14	12	14
PI_2	14	15	13	13
PII_1	13	14	13	13
PII_2	12	13	11	12

4. Conclusions:

From the obtained results the following conclusion can be drown:

The prepared surfactants have good surface properties; they reduce the surface tension of the water and have low CMC. The prepared compounds have strong adsorption on the metal surfaces so they prevent their corrosion in acid medium. The inhibition efficacy is very high in 1M HCl , about 99% for concentration of 2.7 $X10^{-4}$ for PII_2. The optimum dosage for all prepared surfactants is around 3X10-5 M which gives inhabitation efficiency of 96-97%. It is found also that they are good emulsifiers for oil base mud compared with the commercial one.

The prepared surfactants have very good biocidal effect towards the tested gram positive and negative bacteria

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Occupational Health Hazard of Egyptian Employees in Contact with Wastage Nourished Swine

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Abstract: Egyptian swine still they are free nourished on wastages in small herds without veterinary health measures. Because of their omnivore's behavior, pigs are naturally exposed to zoonotic agents in their setting with subsequent direct human occupational hazards. Brucellosis, Leptospirosis and Toxoplasmosis are the major diseases link human exposure for natives in contact with swine. So, updating the sero-prevalence of these pathogens among contact employees reflect to how extent the human bio-hazards are due to direct contact with swine or their contaminant subset. Therefore, sera of 230 free wastage nourished pigs were collected at Cairo, Egypt. Also, 127 serum samples were collected from racing occupational workers. Human and swine sera were serologically analyzed for antibodies against Brucella, Leptospira and Toxoplasma by using commercial kits. Antibodies against *Brucella* were detected in 29/ 230 (12.61 %) of swine sera, and 11/127 (8.66 %) of workers sera by using Rose Bengal plate test. Antibodies against *Leptospira* serovars were detected in 53/230 (23.04%) of swine sera using the microscopic agglutination test (MAT) at a titer of 1:200. The highest seroprevalence was recorded for *L. pomona* (45.28%), followed by *L. grippotyphosa* (33.96%) and *L. icterohaemorrahgiae* (20.75%). The seropositive human sera were 25.9% with the highest incidence corresponding to *L. pomona* serovar (11%). Results of the indirect fluorescent antibody test showed that anti-*Toxoplasma* antibodies were detected in 74.78% (172/230) and 37.79% (48/127) of swine and contact employees respectively. It can be concluded that serological assays concerning brucellosis, leptospirosis and toxoplasmosis verify direct occupational exposure for high risk group's manipulating employees through carrier animals or their pollutant conditions.
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Key words: health hazard, swine, Brucellosis, Leptospirosis, Toxoplasmosis, Egyptian employees.

1. Introduction:

Pigs are omnivores, feed on wastage that grasping insects, rodents, plus different bio-hazards residuals. The swine living situation shear habitats with dogs, cats, rodents and wild birds that maximize their exposure to be reservoirs for various zoonoses. The local Egyptian wastage feeding swine are scattered in small herds without rigid veterinary health measures that signify spots for spreading epidemics. Brucellosis, leptospirosis and toxoplasmosis characterize the most swine's occupational zoonoses that induce swine's abortion and fetus depletion (Samaha et al., 2008).

Swine brucellosis is a worldwide zoonosis symbolize an important source for human brucellosis and is mainly caused by *Brucella suis*, while *Brucella abortus* and *Brucella melitensis* can infect pigs but are only mildly pathogenic (Shimshony, 2009). The zoonotic impacts of those pathogens were designated as select biological agents of Category B by the Centers for Disease Control in Atlanta, USA (Corbell, 2006). Brucellosis causes more than 500 000 human infections per year worldwide (Corbell, 2006). According to WHO (1998), brucellosis is endemic in Egypt**,** *Brucella melitensis* biovar 3 is the most commonly isolated species from Egyptian animals (Refai, 2002). Brucellosis in animals causes tremendous economic losses due to abortion, premature birth, decreased milk production, and reduced reproduction rate (WHO, 2009). Human brucellosis is mainly an occupational risk for farmers, veterinarians, abattoir workers, laboratory personnel, and others who work in contact with animals or their raw products (CDC, 2007).

Leptospirosis has been recognized as an emerging global public health problem because of its increasing incidence in both developing and developed countries (Slack et al., 2008). A number of leptospirosis outbreaks have occurred in the past few years in various places such as Nicaragua, Brazil and India (Health et al., 1965). It is a direct zoonotic disease caused by spirochetes belonging to different pathogenic species of the genus Leptospira. Human infection results from accidental contact with carrier animals or contaminated environment. The primary source of leptospirae is the excretory animal, from whose renal tubules leptospirae are excreted into the environment with the animal urine (Mckiel et al., 1961). The majority of the leptospiral infections is either sub clinical or result in very mild illness and

recover without any complications. Illness develops and progresses rapidly, leading to organ failure and often death if not treated and the case fatality ratio could be about 40% or more. Abortion, occurring 2-4 week before terms is the most common manifestation of swine leptospirosis. Because of the variable manifestations of leptospirosis, it is often misdiagnosed and under-reported (Slack et al., 2008).

Toxoplasma gondii is an obligate intracellular tissue cyst-forming coccidian protozoan with zoonotic impact. the course of disease is generally benign, but during unfit host immune condition through virulent strain, the protozoan stimulate serious affection with significant morbidity and mortality including humans with Acquired Immunodeficiency Syndrome (AIDS) or submitted to corticosteroids and cancer chemotherapy (Jones et al., 2001). The protozoon transmitted vertically via placenta to the fetus by acute stage tachyzoites, while horizontal transmission may involve either ingesting of the environmentally sporulated oocysts that eliminated un-sporulated only via shedder cats or via ingesting the dormant chronic tissue cysts stage in meat of food animals. Diffusion may also occur via tachyzoites through blood transfusion, tissue transplants, un-pasteurised milk or aborted fetal fluids and membrane (Tenter et al., 2000). Humans become infected mainly postnatal by eating raw or inadequately cooked meat containing tissue cysts (Jones et al., 2001). Ingestion of pork tissue cyst signifies one of the most prevalent sources of human toxoplasmosis, because they can remain viable at 52ºC for 9.5 minutes (Aspinall, 2002).There is no possible mode of transmission to human via lively animals in contact including pigs, but manipulating pork confirm occupational risk due to bradyzoites diffusion through skin abrasions (Cook et al., 2000).

Detection of the sero-prevalence of occupational brucellosis, leptospirosis and toxoplasmosis in working personnel in contact with free nourished swine is of ecological impact, reflecting to how extent the human bio-hazards are due to occupational activities. Also, this study confirm the necessitate of public health worry by Egyptian veterinary authorities' toward wastage feeding swine on such unhygienic situation that believed to be spots for expand epidemics.

2. Material and methods:

A. Sample collection:

Blood samples were collected from both 230 pigs and 127 racing occupational workers at Cairo in Egypt. Human and swine sera were separated and stored at -20 until analyzed.

B. Serological tests:

1. Indirect fluorescent antibody test (IFAT) for diagnosis of toxoplasmosis:

The formalized whole tachyzoites antigen slides for the IFAT was prepared as described by Goldman (1957) and the technique was adopted according to the procedures mentioned by Shaapan et al. (2008) and) at a dilution of 1:200 of human and swine sera.

2. Microscopic agglutination test (MAT) for diagnosis of leptospirosis:

Leptospira interrogans serovars *pomona*, *icterohaemorrhagiae*, *grippotyphosa* were used for MAT. They were grown in EMJH liquid and semisolid media (Difco, USA) at 29-30 ºC and the growth was assessed by dark field microscopy regularly. These reference leptospiral strains were kindly obtained from C. Sulzer, C.D.C., and Atlanta, U.S.A. The gold standard serodiagnostic test for leptospirosis is MAT, which was performed as per the method of Galton et al. (1965) and its modification (Cole et al., 1973**)**. Briefly, the sera from the swine were serially diluted from 1:100 to 1:3200 in phosphate buffered saline (PBS), pH 7.2 and allowed to react with live antigen suspensions of the reference leptospiral serovars. After 2 hours incubation at 37 °C, the serum-antigen mixtures were examined by dark field microscopy for the presence of agglutination/ clearance of the organisms and the titers were determined. Reciprocal agglutination titers of greater than or equal to 200 were considered as positive reactions.

3. Rose Bengal plate Test (RBPT) for diagnosis of brucellosis:

The Rose Bengal stained brucella antigen is used for the early detection of brucella agglutinins (*Brucella Suis*) according to (Alton et al., 1988). For RBT, 1 drop (30 ml) of test serum was added to Rose Bengal antigen on a white porcelain plate and mixed thoroughly with a stick. The plate was rocked slowly for 4 minutes and observed.

3: Results:

Swine and in contact human sera which assayed serologically, illustrated sero-positive results with different percents (74.78 & 37.79), (12.61& 8.66) and (23.1&25. 98) corresponding to the *Toxoplasma gondii, Brucella suis* and *Leptospira spp* respectively. The used testes were Indirect Fluorescent Antibody Test, Rose Bengal plate test and Microscopic Agglutination Test consequence to the three zoonoses respectively (Table 1).

Swine and human sera demonstrating wide-ranging results of *T. gondii* antibodies by IFAT titers range from 1/16 to 1/1024. The higher swine

percent (18.60) was established at titer of (1/512), while the higher human percent (22.92) was recognized at titer of (1/128) (Table 2).
Varied percents were detected in swine and human sera (45.3 & 11), (20.8 & 7) and (33.9 & 7.9) corresponding to the *L. pomona, L.icterohaemorrahgiae* and *L. gippotyphosa* respectively. Also, swine and human sera confirming various results of Leptospiral Serovars antibodies by MAT titers range from 1/200 to 1/3200 **(**Table 3).

Table 1: ***Toxoplasma gondii, Brucella suis* and *Leptospira spp* Comparative sero-positive results in humans and swine sera.**

	No. of tested swine sera	No. of sero-positive & (%)	No. of tested Human sera	No. of sero-positive & (%)	Used test
Toxoplasma gondii	230	172(74.78)	127	48(37.79)	IFAT
Brucella suis.	230	29(12.61)	127	11(8.66)	RBT
Leptospira spp.	230	53(23.1)	127	33(25. 98)	MAT

IFAT: Indirect Fluorescent Antibody Test **MAT:** Microscopic Agglutination Test
RBT: Rose Bengal plate test.

Table 2: Human and swine sera comparative results of *T. gondii* detected by IFAT titers.

	IFAT Titers (total immunoglobulin) No of positive cases						
	1/16	1/32	1/64	1/128	1/256	1/512	1/1024
No. of seropositive swine							
172 /230	17	18	19	29	29	32	28
(%)	9.89	10.46	11.05	16.86	16.86	18.60	16.28
No. of seropositive human							
48 /127	2	4	7	11	9	7	8
(%)	4.16	8.32	14.58	22.92	18.75	14.58	16.66

Table 3: Human and swine sera comparative results of Leptospiral Serovars detected by MAT titers.

serovars		1≥200 MAT Titers (total immunoglobulin)				
		1/200	1/400	1/800	1/1600	1/3200
	53/230 Seropositive swine & (%)					
L. pomona	24(45.3)	3	4	6	6	5
L.icterohaemorrahgiae	11(20.8)	1	2	3	2	3
L. gippotyphosa	18(33.9)	2	3	4	6	3
Total	53(23.1)					
	33/127 Seropositive persons & (%)					
L. pomona	14(11)	2	3	3	5	1
L.icterohaemorrahgiae	9(7)	3	3	2	1	0
L. gippotyphosa	10(7.9)	4	2	1	3	0
Total	33(25.9)					

4. Discussion:

Infected pregnant sows may be aborted consequence to infection by Brucella, Leptospira or Toxoplasma, their foeti, fetal fluid, and membranes are harboring the causative pathogen. So, the aborted swine constitute direct occupational health hazard for manipulating employees or animals that licked or eaten the infected fluids or tissues. Accordingly, the three pathogens could maximize their persistence in infected swine's herds. Apparent healthy sero-positive swine may be chronic shedders for Brucella and Leptospira via urine and other body fluids, while *T. gondii* sero-positive swine may harbor tissue cysts which don't usually constitute occupational bio-hazard to human, except through manipulating or feeding under cooked pork.

In this study, 12.61% and 8.66% of the examined swine and contact persons sera had Brucella antibodies, the compatibility between swine's and human percentages are due to the fact that Brucella is of highly contagious characters. Where humans in contact mainly contracted infection from shedder swine, and usually predisposes farmers, shepherds, butchers, laboratory workers,

veterinarians and slaughterhouse workers. Also, indicate that swine's behavior in clay water pools may play vital role for swine's and human communication via droplet infection and through intact or abraded skin. The practical confirmation was done when three occupational groups exposed to brucellosis hazard were investigated in pork in 1967, using three standard tests. of 80 veterinarians, 92·5% had serological evidence of past or present brucella infection **(**Pappas et al., 2006). On the other hand, lower results of brucella antibodies in human were recorded by Omer et al. (2002) who found that the prevalence of brucellosis among high risk occupational groups using Rose Bengal test is among occupational personnel (4.5%). Mudaliar et al. (2003) recorded prevalence of brucellosis of 5.33% in animal handlers and advised that the clinician should keep in mind the possibility of an occupational or environmental exposure in cases of fever of unknown origin. The countries with the highest incidence of human brucellosis are Iran (29.8/100,000), Saudi Arabia (32.8/100,000), Syria (21.0/100,000), Jordan (20.4/100,000), Palestine (21.5/100,000) and Oman (16.6/100,000). Bahrain and Cyprus have reported zero incidences. In the rest of the countries, the incidence varies from 0.8/100,000 in Egypt to 9.0/100,000 in Tunisia (Smits and Culter, 2004).

Concerning leptospirosis, in this study, the percent of infected swine and contact humans are compatible 23.04 and 25.98, respectively. The higher incidence in workers may be clarified due to the fast changeability in swine's herd individuals, in contrast to long term stability with the same occupational employees. Nei and Kumar (2000) investigated the sera from 1215 meat inspectors and 1248 meat workers for the presence of agglutinating titers of 1:24 or greater to the serovar , known to be endemic in New Zealand. Although 10 percent of meat inspectors and 6.2 percent of meat workers were seropositive, only 9.5 percent of meat inspectors and 4.1 percent of meat workers had titers compatible with occupational exposure to domestic stock, although the results of this survey demonstrate that leptospirosis is a definite occupational hazard in the meat industry, the risk is threefold less than for dairy farm workers and pig farmers (Slack et al., 2008). Infection is usually due to contact with the pig's or other wildlife urine. Rodents play a significant role in disease maintenance within confinement operations and other swine facilities (Levett, 2001). Venereal transmission from carrier boars and sows may play a role in maintenance of the disease (Bharti et al., 2003).

In the present investigation, high percent (74.78) of the examined swine sera are carried *T. gondii* antibodies and could be regarded as high risk animal groups for both public and animals' health, connected to the pattern of wastage raising swine's on oocysts dirty unsanitary condition. The prevalence usually higher in sows that suffers toxoplasmic abortions due to placental transmission or postnatal infection via licking aborted foeti and amniotic fluids containing tachyzoites,plus congenital transmission may occur during pregnancies (Dubey, 2002). Swine feed from the ground; consequently the high sero-positive percent in free range swine is accepted as significant bio-indicator evaluate the degree of *T. gondii* oocysts environmental pollution, and reflect the fragile measures opposite to stray cats in the locality of swine subset (Howe et al., 1997). Hassanain et al., (2008) confirm high oocyst Egyptian environmental pollution through high incidence of naturally infected kittens (70.6 %) with consequence shedding oocyst. Also, ELfadaly, (2007) confirm 61.4 % sero-positive Egyptian sheep fed on the same unhygienic condition, The difference between swine and sheep species may be related to the omnivorous behavior of swine that usually feed on rodent, meat or poultry residuals containing tissue cysts, this in contrast to herbivorous sheep.

Inadequate rodent control is considered to play a role in swine toxoplasmosis. Three organic pig farms with known rodent infestation were included in study conducted by Fuentes et al. (2001). On these farms, presence of *T. gondii* in trapped rodents was evaluated by real-time PCR. All rodent species and shrews investigated had *T. gondii* DNA in brain or heart tissue. Prevalence was 10.3% in Rattus norvegicus, 6.5% in Mus musculus, 14.3% in Apodemus sylvaticus and 13.6% in Crocidura russula. Initial *T. gondii* seroprevalence in the slaughter pigs was dropped on the three farms from17% to 8% after rodent control.

Pigs are considered to be the most important meat source of *Toxoplasma gondii* for humans in the United States (Grigg and Boothroyd, 2001). Antibodies to *T. gondii* were found in 16.97% (141/831) with slaughter pigs having the highest rate (22.28%), followed by breeding sows (16.59%) (Ajzenberg et al., 2002). During the hunting seasons 2002-2008 from wild boar in France, Antibodies to *T. gondii* were found in 26 (17.6%) of 148 wild boars using the modified agglutination test (MAT, positivity threshold: 1:24). Seroprevalence was 45.9 % (Cook et al., 2000). Prevalence of *T. gondii* in market pigs appears to have declined in the US with the advent of improved sanitation in large production facilities (Davies et al., 1998). However, a serological survey of pigs of variable age from 85 New England farms showed an overall prevalence of 47%, with 91% of the herds having at least one

seropositive pig, and within-herd prevalence varied between 4 and 100% (Gamble et al., 1999).

In the present work, swine's that carrying *T. gondii* antibodies were 74.78 %, while 37.79% of occupational humans in contact. The difference in percent between swine and working persons was referred to that *T. gondii* is not mainly transmitted via occupational mode, unless through skin abrasions during handling aborted foeti harboring tachyzoites or pork containing bradyzoites.

Egyptian swine's included in this study found to be apparent health when blood samples were taken, although they are still free feed on wastages; perhaps confirming the belief that, in pigs, equilibrium exists between zoonotic agents and swine species. It can be concluded that serological assays concerning brucellosis, leptospirosis and toxoplasmosis verify direct occupational exposure for high risk group's manipulating employees through carrier swine's or their pollutant conditions. Also, this study reflect the need of public health worry by Egyptian veterinary authorities' toward wastage nourished swine's on such condition that costumes spots for spread out epidemics .

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Study of medical plant distribution in Lasem area of Northern Iran

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Abstract: In order to gather and identify the medicinal plants at the mountainous rangelands of Lasem in Larijan of northern Iran, the field survey method was done. The results showed that there were 42 medicinal species in the area belonging to 18 classes. The classes Rosaceae with 8, Compositae with 8, and Labiateae with 7 species had the biggest number of medicinal species; and the growth forms hemicryptophyte and trophyte were the most common. Furthermore, leaves and flowers were the main plant parts used, essence and tannin were the most common compounds, and the most common curative effect was as diuretic. The types, features, and the compounds found in the medicinal plants of this ecosystem suggest that this region has a high potential with regard to the production of medicinal plants; and if the exploiters of the rangelands get to know this potential, they will be able to maintain the ecosystem, to keep it sustainable, and to reap huge economic benefits as well.

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Key words: Chemical compounds, Curative effects, Lasem, Medicinal plants

1. Introduction

Rangelands are considered a renewable natural resource which covers 43% of the land area of the planet earth; and they occupy 54.6 of the land area of our country as well. Rangelands, besides having a protective value (in protecting soil and water) and producing forage (animal products) and protecting the environment and serving as a genetic source, etc, enjoy a special status as regards the production of medicinal and industrial plants. Therefore, acquaintance with and study of the vegetative cover is necessary in this regard; and is an important step in making multiple use of rangelands (Hosseini 2001).

It can be easily seen that the main factor causing the destruction of rangelands and the expansion of deserts is the utilization of the basic products of rangelands in the framework of raising livestock. Many farmers and livestock producers see rangelands as a source of forage and continuously increase the number of stock kept on rangelands which, considering the lack of proper management of rangelands ,has intensified the process of the destruction of rangelands. Therefore, acquaintance with and utilization of the other pasture products can have a fundamental role in improving the economic situation of exploiters of rangelands and in preventing pressure on the vegetative cover.

Medicinal plants are among the most important by-products of rangelands which unfortunately, despite the fact that they have a high economic efficiency and that they are easily extractable and contrary to the situation in other countries, have received less attention in our country; and still many of our people do not know much about the types, the benefits, the composition, and the curative features of these plants (Nazir et al., 2010). Therefore, we studied the region of Larijan, especially the sub-region of Lasem, because of the important medical and economic values of medicinal plants as a tool for sustainable exploitation of the mountainous rangelands of Alborz, and due to the fact that this sub-region has not been studied with regard to medicinal plants because of its peculiar climatic and topographic features ,and also for the reason that the farmers and livestock owners who use these rangelands do not have sufficient knowledge about this green treasure trove .

Shahrokhi (2001) named local knowledge of medicinal plants an effective factor for sustainable development, and mentioned the protection of the environment, the production of raw materials, the

creation of jobs, and the access to the international market as some of the factors that, through the use of medicinal plants, have a role in sustainable development. Ahmadi (2001) gathered and identified 96 species of medicinal plants in the province of Lorestan and showed that most of them belonged to the classes of Liliaceae, Asteraceae, Laminaceae, and Rosaceae. Akbarzadeh (2003) and identified 36 species and 18 genera of the mint family and determined the distribution of each of the species as well.

Lebaschi et al. (2004) studied the growth status of the medicinal plants of salvia, milfoil, spogel seed plantain, marigold, and chamomile and stated that salvia and milfoil grew best in dry conditions; and can be established as medicinal plants resistant to dry conditions or to limited supply of water. Mazandarani et al. (2004) introduced 107 medicinal plants from the mountain of Ziarat in the city of Gorgan belonging to 103 genera and 42 families; and found out that the most important life forms in the region were the geophytes (36.4%), the trophytes (20.6%), the fanerophytes (19.6%), the camophytes (12.1%), and the hemicryptophytes (11.2%).

Zarezadeh et al. (2005) studied 37 species of drought resistant medicinal plants in the province of Yazd and found out that the families Apiaceae, Laminaceae, and Solanaceae had the most number of species. Fallah hosseini et al. (2005) investigated the effects of citrule in reducing blood sugar in type two diabetics and concluded that prescribing 100 milligrams of this plant three times a day reduces blood Hb1Ac and blood sugar without any side-effects on the digestive system, the liver, or the kidneys. They also carried out another study on the medicinal effects of onions, garlic, bitter cucumber, fenugreek, saltwort, whortleberry, blessed milk thistle, and green tea on diabetes. Mirza et al. (2005) conducted a research titled Identification and study of the chemical compounds in the essence of *Lepidium sativum* and stated that from among 25 identified compounds in this species, which constituted 94.7% of the essence, camphor was the main one with 19.8%.

Jalali et al. (2006) used different methods to compare the anti-listerial effects of thyme, eucalyptus, chamomile, rosemary, and salvia and concluded that that only the hydro-alcoholic extract of eucalyptus had anti-listerial effects on Listeria monocytogenes and can be considered as an anti-listerial compound. Jamshidi et al. (2006) introduced thyme as one of the valuable plants found in Alborz heights and showed that the essence of this plant had a high percentage of Thymol and Carvacrol and the best habitat for it to produce the highest yield and the best quality crop is at an altitude of 2400 meters above sea level. Akbarinia et al. (2006) studied the medicinal plants of the province of Ghazvin with regard to their biological and floristic features. They identified 85 medicinal species belonging to 33 classes and 76 genera, with the classes Laminaceae with 21 and Asteraceae with 6 species being the most important classes in the region.

Bah et al. (2004) showed that steroidal saponins, alkaloid glycosides and vitanoloidal steroids are the secondary metabolites found in the genus Solanum which have anti-cancer effects in people. Thabrew et al. (2005) investigated the anti-toxic effects of the species Hemidesmus indicus, Smilax glabar, and Nigella sativa, and showed that they have a very high anti-cancer potential because cancerous cells injected with the extract of these plants died after 24 hours.

Mathabe et al. (2006) investigated the medicinal plants in the region of Limpopo in South Africa and reported the anti-bacterial features of the species of *Schotia brachypetala* and *Punica granatum* in curing diarrhea. Lee et al. (2007) studied the antioxidant features of 45 medicinal species and showed that species such as *Scutellaria baicalensis* and *Fraxinus rhynchophylla* are a rich and natural source of these compounds.

Yun-long et al. (2007) investigated the insecticidal effects of the species *Matricaria chamomilla* and *Thymus vulgaris* and showed that the compound thiacloprid was the insecticide in these plants which decomposed rapidly. Yinger et al. (2007) studied the medicinal plants at Bale Mountains National Park in Ethiopia and referred to their effects in curing animal diseases. They identified 74 species belonging to 64 genera and 27 families of medicinal plants, and reported that forbs (47.3%) and bushes (37%) were the most common growth forms exploited, the roots and leaves of which were used most of all .

1. 2. Materials and Methods: The Region Studied

The area studied in the summer rangelands of Lasem, which is one of the sub-regions of the Talar River watershed, has a longitude of 52º 75’ 10” to 52º 84’ 15” and a latitude of 35º 92’ 34” to 35º 99’ 45”, an area of 3450 hectares, and its altitude varies from 1900 to 3400 meters above sea level. The average yearly temperature is 11 degrees centigrade and the average yearly rainfall is from 450 to 550 millimeters. The climate of the area varies from semi-humid at low parts

to semi-arid at high parts. The main formations are the geological formations of Lar and Shemshak.

2.1. Methodology

First, we used aerial pictures with a scale of 1: 20000 and topographical maps with a scale of 1: 50000 to locate Lasem, and then used the field survey method to study and sample the medicinal plant cover in the region. The samples were identified using the colored Flora and the herbarium at the Natural Resources College. Then we referred to valid scientific references, consulted with experts at research centers, and used local knowledge to determine features such as chemical composition, curative effects, the parts of plants used, and the distribution of various species. Finally, the Exel software was used to analyze the data; and the final adding-up was performed. It must be mentioned that important information about medicinal plants, their effects, and the way they are used was obtained from local people; and this information played a key role in the analysis of data.

2. 3. Results

3.1. Results Drawn from the study of the Flora of the Region

The medicinal species identified in the region studied belonged to 18 classes and 42 genera (Table 1). The classes Rosaceae with 8, Compositae with 8, and Labiateae with 7 species had the most medicinal species in the region; and 12 classes each with one species were the smallest. Figure 1 shows the abundance of plant species. We also determined the growth forms of the medicinal plants in the region and found that hemicryptophytes with 55% and Trophytes with 21% constituted the most common growth forms in the plants in the region. The results concerning the growth forms are shown in Figure 2.

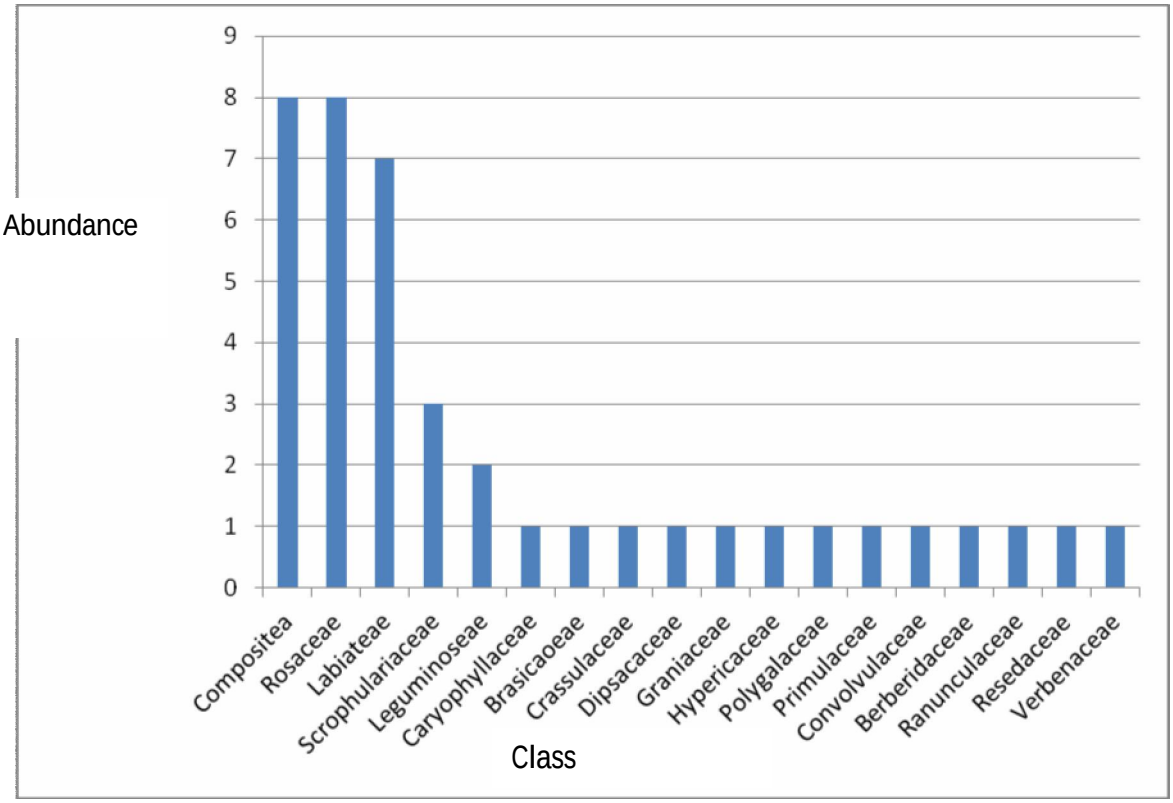

Figure 1- Abundance of Medicinal Plant Families in the Region

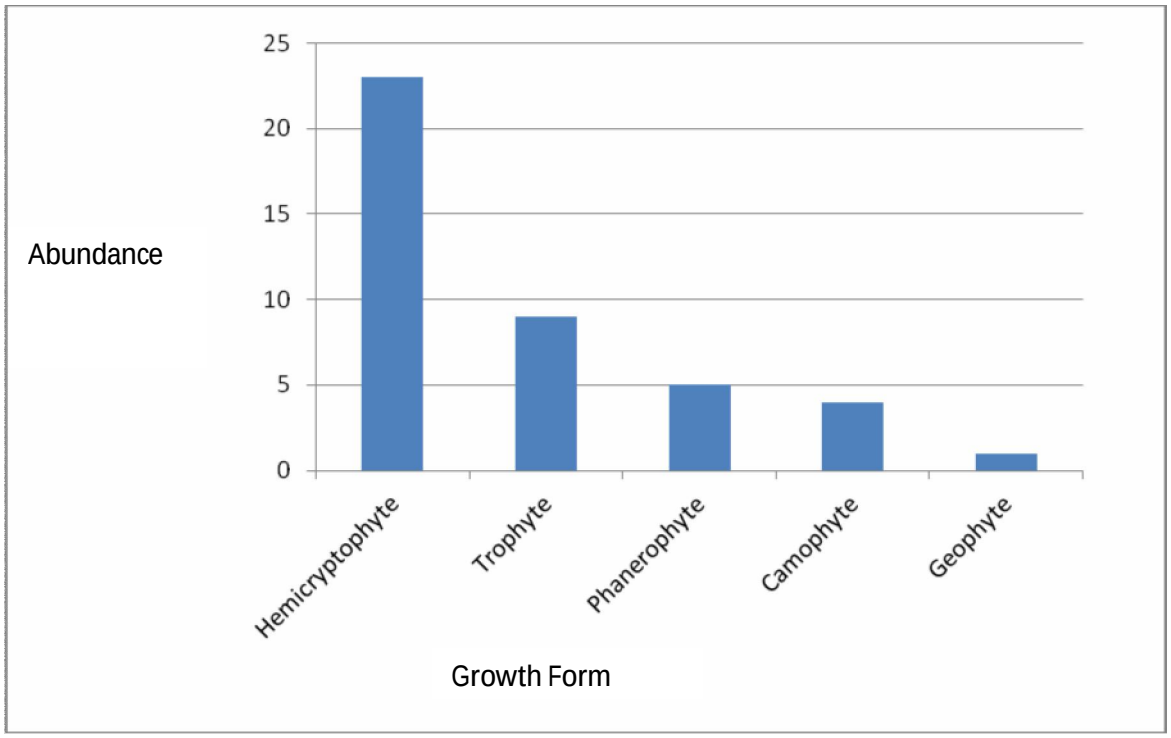

Figure 2 – The Growth Forms of the Medicinal Plants in the Region

Table 1. The list of scientific name, class, growth form and life form of the determined plants.

Ser. no	Scientific name	Class	Growth form	Life form
1	*Achillea spp*	Compositeae	hemicryptophyte	perennial
2	*Anthemis spp*	Compositeae	hemicryptophyte	perennial
3	*Artemisia absantinium*	Compositeae	hemicryptophyte	perennial
4	*Astragalus gossypinus*	Leguminoseae	hemicryptophyte	Perennial
5	*Berberis vulgaris*	Berberidaceae	Camophyte	Perennial
6	*Capsella bursa pastoris*	Brasicaceae	Trophyte	Annual
7	*Centaura virgata*	Compositeae	hemicryptophyte	Perennial
8	*Convolvulus arvensis*	Convolvulaceae	Trophyte	Annual
9	*Crataegus microphylla*	Rosaceae	fanerophyte	perennial
10	*Delphinium elbursense*	Ranunculaceae	hemicryptophyte	annual
11	*Digitalis nervosa*	Scrophulariaceae	hemicryptophyte	perennial
12	*Echinops cephalotes*	Compositeae	Hemicrtptophyte	Perennial
13	*Fragaria vesca*	Rosaceae	Hemicryptiphyte	Annual
14	*Granium tuerosum*	Graniaceae	Hemicryptophyte	Perennial
15	*Hypericum*	Hypericaceae	Hemicryptophyte	Perennial
16	*Hyssopus angustifolius*	Labiateae	hemicryptophyte	Annual
17	*Lamium album*	Labiateae	Hemicryptophyte	Perennial
18	*Malus commonis*	Rosaceae	Fanerophyte	Perennial
19	*Medicago sativa*	Leguminoseae	Hemicryptophyte	Perennial
20	*Mespilus germanica*	Rosaceae	Fanerophyte	perennial
21	*Nepeta crassifolia*	Labiateae	Hemicryptophyte	Perennial
22	*Nepeta racemosa*	Labiateae	Hemicryptophyte	Perennial
23	*Origanum vulgar*	Labiateae	Trophyte	perennial

24	*Polygala platyptera*	Polygalaceae	Hemicryptiphyte	Perennial
25	*Potentilla reptense*	Rosaceae	Hemicryptophyte	Perennial
26	*Primula acaulis*	Primulaceae	Hemicryptophyte	Perennial
27	*Pronus divaricata*	Rosaceae	Fanerophyte	Perennial
28	*Pyrus boisseriana*	Rosaceae	Fanerophyte	Perennial
29	*Reseda lutea*	Resedaceae	Hemicrtptophyte	Biennial
30	*Rosa persica*	Rosaceae	Camophyte	Prennial
31	*Scabiosae amaena*	Dipsacaceae	Hemicryptophyte	Perennial
32	*Sedum rubense*	Crassulaceae	Trophyte	Annual
33	*Sencio vernalis*	Compositeae	Geophyte	Annual
34	*Silene pruinosa*	Caryophyllaceae	Trophyte	Annual
35	*Stellaria media*	Caryophyllaceae	Trophyte	Annual
36	*Taraxacum monthanum*	Compositeae	Trophyte	Annual
37	*Teucrium polium*	Labiateae	Camophyte	Perennial
38	*Thymus pubscense*	Labiateae	Camophyte	Perennial
39	*Tragopogon marginatus*	Compositeae	Trophyte	Annual
40	*Verbascum specium*	Scrophulariaceae	Hemicryptophyte	Perennial
41	*Verbena officinalis*	Verbenaceae	Hemicryptophyte	Perennial
42	*Veronica persica*	Scrophulariaceae	Trophyte	Annual

3.2. Results Drawn from Information Supplied by Local People

Since the field survey method was used in our study, we also gathered information from some local people about the medicinal plants, such as their benefits and their effects. Some of the most important of this information is shown in table 2.

Table 2: Information about important local medicinal plants

Important Medicinal Species	**The Parts of the Plants Used**	**The Most Important Medicinal Effects**
Pyrus cordata	Bark, stem, leaves and fruit	Astringent ,sedative , reduces uric acid
Barberry	Bark, roots, stem	Purifies blood, cure for digestive problems and for sore throat
Crab apple	Bark, stem, leaves, and fruit	Astringent, sedative, reduces uric acid
Hawthorn	Flowers and fruit	Anti-spasm, heart tonic
Medlar	Leaves and fruit	Cure for sore throat and diarrhea
Polygala	Roots	Expectorant, cure for coughs
Camomile	Leaves and flower bearing browses	Anti-spasm, cure for worms
Milfoil	Leaves and flower bearing browses	Sedative for nerves, cure for diarrhea,general body tonic
Thyme	Leaves and flower bearing browses	Regulates the nervous system and blood circulation , tonic
Salsify	roots	Cure for skin diseases , appetizer

3.3. Chemical composition of Medicinal Plants

Studies carried out about the chemical compositional of medicinal plants showed that essence and tannin with 30% and 26% respectively, were the most frequent and ether, mucilage and pigments with 4 % , 4% , and 3% were the least frequently found compounds in the medicinal species in the region (Figure 3).

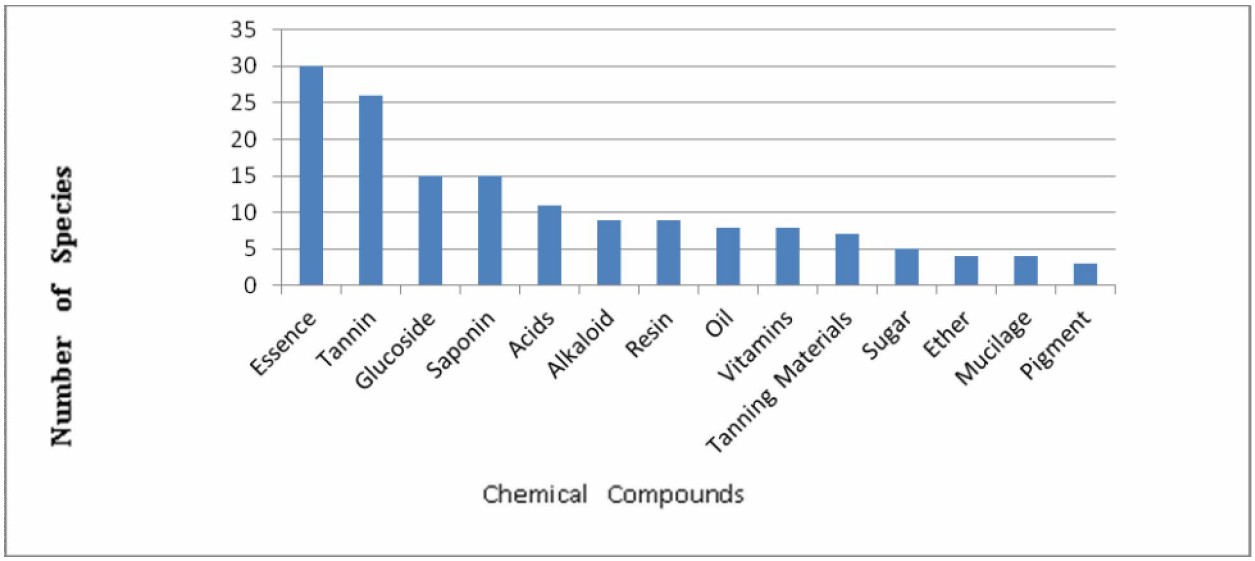

Figure 3: Chemical Compounds in the Plants of the Region of Lasem

3.4. The parts of the medicinal plants used

Different parts of leaves, flower bearing browses, roots, flowers, shoots, and fruits are the parts of medicinal plants in the sub-region of Lasem used for curing diseases and for conventional medicinal purposes, the flowers and the leaves being the parts most used (Figure 4).

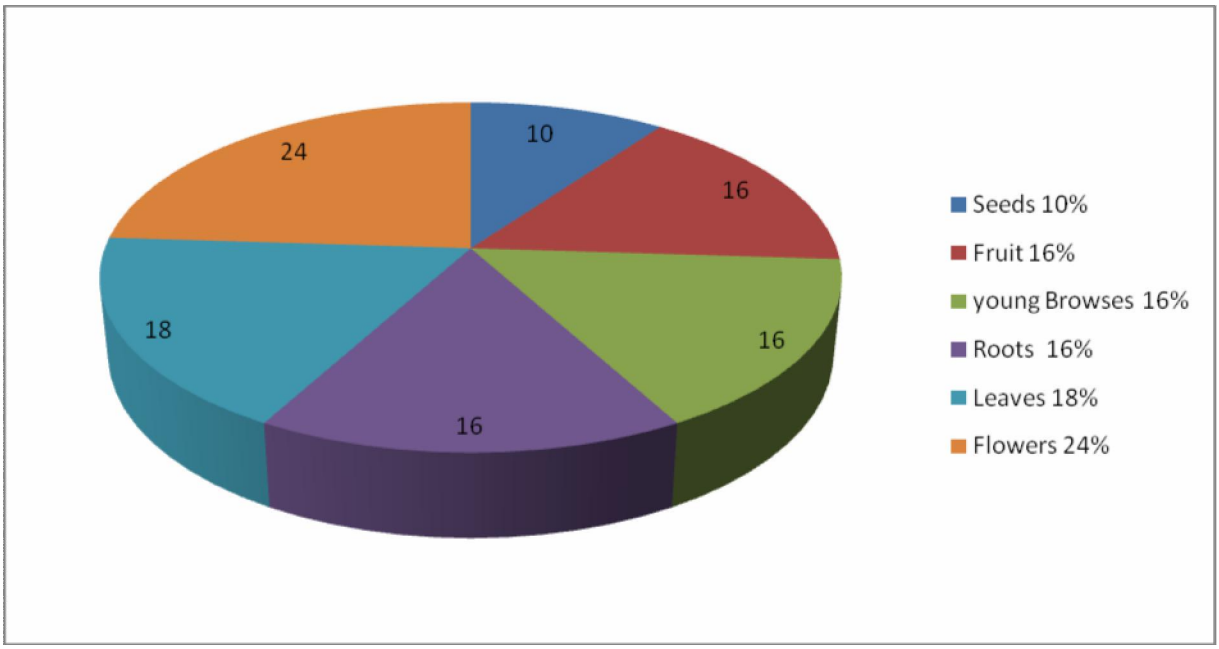

Figure 4: Abundance of the Parts of Medicinal Plants Used in the Region of Lasem

In investigations about the curative effects of the medicinal plants in the region of Lasem, it was found that most of these species had curative effects (Figure 5).

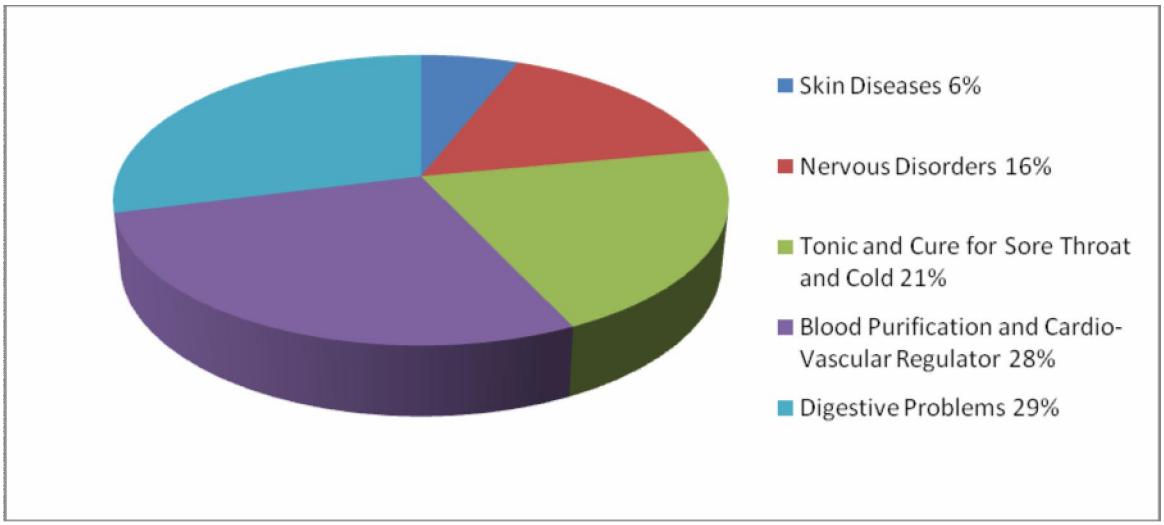

Figure 5: Curative Features of the Plants in the Region of Lasem

4. Discussion and conclusions

Given the results obtained, the abundance of the species of the family Labiateae and also the abundance of species containing essence and tannin in the sub-region can be ascribed to the fact that these species are not grazed because stock mainly do not graze plants containing these compounds; and hence plants containing them have become more abundant. On the other hand, the abundance of the growth forms Hemicryptophyte and Trophyte appears logical since these plants are more resistant to adverse environmental conditions, especially grazing, and also because trophytes are adapted to short growing seasons common in the cold, mountainous rangelands.

Since the grazing pressure on rangelands is mainly due to economic incentives which exist for those who exploit rangelands, and because the region of Cherat has a tremendous potential for the production and multiplication of medicinal plants, attention to these huge resources can, besides preserving the diversity of life forms which is the basis for the survival of natural ecosystems, will be useful in improving the economic situation of livestock producers; and by correctly training them in properly exploiting these plants, and also through cultivating and multiplying these species it is possible to reduce the pressure of excessive exploitation of the vegetative cover. Results obtained from the study of the curative features of these plants show that there is an abundance of plants with astringent and purgative effects which, considering the prevalence of diseases of the digestive system in the rural parts of the sub-region of Lasem, signifies the importance of identifying these species and the need for the pasture exploiters to become acquainted with these plants. Moreover, the abundance of plant species effective in purifying blood and in strengthening the heart, and the familiarity of the local residents with these plants, has resulted in fewer incidences of cardio-vascular diseases among these people. Furthermore, since flowers are the part most used in these species, and because flowers play the major role in apiculture, it is possible to prepare the grounds for the development of apiculture, which has been practiced in the region before, along with the cultivation and multiplication of medicinal plants, so that, while preserving the ecologic values of the sub-region, multiple use of these diverse rangelands and improving the economic situation of the local people and domestication of the existing plant species can be achieved. Therefore, familiarizing the pasture exploiters with the existing medicinal species and training them in the format of medicinal plants, as a practical strategy in raising the spirit of cooperation among them will create jobs and economic profits for pasture exploiters and will also greatly help in sustaining this natural ecosystem.

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18.

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Protective Effect of Taurine and Bismuth Subnitrate against Cyclosporine and NSAID-induced Nephrotoxicity in Rats.

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Abstract: The immunosuppressive drug cyclosporine (CSA) has been successfully used in several diseases with immunological basis and in transplant patients. Nephrotoxicity is the major limitation for CSA use. Recent evidence suggests that reactive oxygen species (ROS) play an important role in mediating CSA-induced nephrotoxicity. Co-administration of CSA and non steroidal anti-inflammatory drug (NSAID), sodium diclofenac (SD), increases the efficacy for pain relief in patients with rheumatoid arthritis. However, clinical studies showed enhancement of cyclosporine nephrotoxicity. To characterize biochemical parameters of nephrotoxicity, the study assessed the effect of CSA (10 mg/kg B.wt) alone or in combination with SD (10 mg/kg B.wt) for 6 weeks on serum creatinine (S.Cr), blood urea (BU), alkaline phosphatase (ALP), total protein (TP), albumin and gamma glutamyl transferase (GGT). Oxidative stress was also evaluated; lipid peroxide measured as malondialdehyde (MDA), lactate dehydrogenase (LDH), as well as oxidized and reduced glutathione (GSSG and GSH) in serum of adult albino rats. CSA alone caused significant rise in BU and S.Cr, serum ALP and GGT, while reduction of serum TP and albumin was observed. In addition CSA also alternated oxidative stress through increasing levels of serum MDA, LDH and GSSG and decreasing levels of GSH and GSH/GSSG ratio. When SD combined with CSA, it enhanced all biochemical parameters of CSA-induced nephrotoxicity. The study also extended to evaluate and compare the protective effect of taurine, (tau), which is a major intracellular free beta-amino acid and potent endogenous antioxidant with Bismuth subnitrate (BSN), an antiulcer drug and a specific inducer of renal metalothionine (MT), against nephrotoxicity induced by CSA and SD administration. The present investigation showed that co-administration of both BSN and taurine could antagonize most of CSA negative effects, by attenuating renal dysfunctions, reducing serum MDA and counteracting the deleterious effects of CSA on oxidative stress markers.
[Suzan F.I. Elsisi, Salwa Kamal El –Nabarawy. **Protective Effect of Taurine and Bismuth Subnitrate against Cyclosporine and NSAID-induced Nephrotoxicity in Rats**. Journal of American Science 2011;7(1):912-921]. (ISSN: 1545-1003). http://www.americanscience.org.

Key Words: Nephrotoxicity, drug interaction, reactive oxygen species.

1. Introduction:

Cyclosporine A (CSA), a cyclic undecapeptide of fungal origin, is the most widely immunosuppressive drug in organ transplantation and in the treatment of autoimmune disorders (Capasso *et al.*, 2008; Shu *et al*., 2009). However, its clinical use has been hampered by frequent reports of nephrotoxicity. In fact, moderate to sever renal dysfunction has been documented in ~30% of CSA-treated patients (Galletti *et al.*, 2005). Although the mechanisms of nephrotoxicity are not completely defined, there is evidence that suggests the role of ROS in its pathogenesis. It has been demonstrated in numerous *in vivo* and *in vitro* experiments that CSA induced renal failure and increased the synthesis of ROS and lipid peroxidation products in the kidney. These include: Upregulation of the cytochrome P450-dependent system in kidney (Buetler *et al.* 2000); perturbation of the balance between vasodilatation-vasoconstriction, which in turn is responsible for tubular hypoxia-reoxygenation (Burdmann *et al.* 2003); increased formation of renal thromboxane A2 and induction of nitric oxide production (Burdmann *et al.*, 2003); direct interference of CSA with intracellular homeostasis of glutathione (Galletti *et al*., 2005). Oxidative stress is the main mechanism resulting in cyclosporine-induced nephrotoxicity because of its ability to stimulate endogenous melatonin production (Ghorbanihaghjo *et al.*, 2008).

Although CSA has been shown in a series of controlled trails to be of benefit, patients continue to require NSAID, as SD, for relief of joint pain and stiffness. The adverse effects of SD include gastrointestinal complaints, liver damages, acute and chronic nephrotoxicity, heart attack, bone marrow depression. SD produces multiple undesirable renal effects. Most of these effects are attributed to inhibition of the synthesis of renal prostaglandin which influences cortical blood flow, glomerular filtration rate and salt and water excretion (Griffin *et al*., 2000). Long term treatment of NSAID leaded to ulceration and gastrointestinal bleeding. Hence most patients used to receive ulcer-protection drugs as bismuth subnitrate (BSN) during long term treatment

with SD. BSN can also protect kidney against toxicity as it is a specific inducer of renal metalothionine (Kondo *et al*., 2004).

Taurine is a sulfur containing amino acid; it is the major intracellular free ß-amino acid that plays various important physiological roles including osmorgulation, bile acid conjugation, viability and prevention of oxidant-induced injury in many tissues (Erdem *et al.*, 2000). The beneficial effects of taurine as an antioxidant in biological systems has been attributed to its ability to stabilize biomembranes, scavenge ROS and reduce the lipid peroxidation (Erdem *et al.*, 2000).

The present study aimed to ascertain how often nephrotoxicity by CSA is associated with concurrent additive nephrotoxicity by SD as well as to investigate and compare the possible protective effect of BSN and taurine against nephrotoxicity induced by concurrent administration of CSA and SD.

2. Materials and methods:

Animals

56 Female Albino rats weighing 120 ± 20 g were used. The rats were obtained from animal breeding laboratory of National Organization of Drug Control and Research (NODCAR) Giza, Egypt. They were kept under strictly hygienic conditions. Rats were put on a standard basal diet and allowed free access to drinking water.

Handling and usage of animals was carried out according to guidelines of institual health care and usage committee of animal lab of NODCAR.

Materials

- Bismuth subnitrate and taurine were purchased from Sigma Co. USA.
- Cyclosporine A and Sodium Diclofenac, were purchased from Egyptian market pharmacy.
- Drug doses were freshly prepared before administration dissolved in water (except BSN which was dissolved in citrate solution) and given orally.

Experimental design

Rats were classified into 7 equal groups each comprises 8 rats and treated daily for 6 weeks, as follow:

G1; Negative (-ve) control group (CON), fed on basal diet.

G2; Taurine (+ve) control group (T), orally administrated 500 mg/kg B.wt of taurine.

G3; BSN (+ve) control group (B), orally administrated 15 mg/kg B.wt of BSN.

G4; Cyclosporine group (CSA), orally administrated 10 mg/kg B.wt of CSA.

G5; Combined-treated group (CSA+SD), orally administrated 10 mg/kg B.wt of CSA plus 10 mg/kg B.wt of diclofenac.

G6; Taurine-treated group (C +T), treated as in G5 and supplemented with 500 mg/kg B.wt of taurine.

G7; Bismuth -treated group (C +B), treated as in G5 and supplemented with 15 mg/kg B.wt of BSN.

At the end of the treatment schedule, blood samples were taken from each rat and let stand to get serum and then rats were sacrificed. Kidney tissue was removed; part of it was subjected to histopathological examinations as described by Bancrofet *et al*. (1996) and the other was homogenized in iced 10% KOH and centrifuged at 5000 rpm for 5 minutes. Supernatants were separated. Serum and supernatants of tissues were processed for the biochemical analyses; S.Cr determined by the method of Houot (1985), BU by Patton and Cruoch (1977), ALP by Roy (1970), TP by Henry (1964), albumin by Doumas *et al.* (1971) and GGT by Szasz (1974), oxidative stress lipid peroxides measured as MDA, GSSG and GSH were determined by HPLC methods of Karatepe (2004); Jayatilleke and Shaw (1993) respectively. LDH was determined by the commercial kits of Buhl and Jackson (1978).

Statistical analysis

Data were presented as mean ± SE. one way ANOVA followed by LSD test were used to evaluate significant differences from different treatments (It was done using SPSS, version 11.5)

3. Results and Discussion:

The nephrotoxicity of CSA remains the major limitation of this widely used immunosuppressive drug. Increased level of free oxygen radicals may play an important role in pathogenesis of its adverse effect. Immunosuppressive action of CSA is mediated by formation of a complex with cyclophilin, which in turn inhibits the activity of protein phosphatase 2B calcineurin. Hence, CSA is also known as calciuneurin inhibitor (Naesens, *et al*., 2009).

The effect of CSA on kidney function and oxidative stress

Treatment of rats with CSA for a period of 6 weeks resulted in a significant ($P<0.05$) increase in BU and S.Cr levels (Table 1 &Fig. 1), suggesting the occurrence of renal dysfunction. These results were in agreement with the earlier investigators, who reported significant alteration in BU, S.Cr in patients and experimental animals after CSA treatment (Mason, 1990; Tariq *et al*. 1999; Khan *et al.*, 2006).

In order to investigate the role of ROS of CSA induced-toxicity, data depicted in table (2) show that CSA produced a marked significant ($P<0.05$) increase in lipid peroxides measured as MDA,

Table (1): The effect of taurine and bismuth subnitrate on blood urea (mg/dl), serum creatinine (mg/dl), total protein (g/dl), Albumin (g/dl), alkaline phosphatase (U/L) and gamma glutamyltransferase (U/L) against cyclosporine and Sodium diclofenac- induced nephrotoxicity in ♀ rats after 6 weeks of treatment.

Animal group		CON	B	T	CSA	CSA+SD	C+B	C+T
Tested Parameters	BU	36.30 ± 1.6	38.76 ± 1.31	39.51 ± 1.53	52.78 ± 2.41*	60.49 ± 1.2*[a]	43.88 ± 1.91[b]	41.55 ± 1.75[b]
	S.Cr	0.70 ± 0.04	0.69 ± 0.04	0.77 ± 0.03	1.46 ± 0.05*	1.80 ± 0.06*[a]	0.89 ± 0.05*[b]	1.02 ± 0.03*[b]
	TP	6.06 ± 0.02	6.07 ±0.03	6.04 ± 0.02	5.14 ± 0.04*	4.82 ± 0.11*[a]	5.44 ± 0.02*[b]	5.50 ± 0.03*[b]
	Albumin	3.36 ± 0.03	3.32 ± 0.03	3.34 ± 0.04	2.70 ± 0.04*	2.20 ± 0.05*[a]	3.13 ± 0.04[b]	3.11 ± 0.03[b]
	ALP	60.73 ± 1.28	64.96 ± 1.9	61.00 ± 2.06	84.8 ± 2.1*	100.40 ± 2.00*[a]	74.50 ± 2.6[b]	73.20 ± 1.96[b]
	GGT	5.42 ± 0.61	5.87 ± 0.53	5.67 ± 0.59	9.32 ± 0.44*	13.42 ± 0.69*[a]	7.08 ± 0.61[b]	7.18 ± 0.56[b]

Significant difference *vs.* CON group: *P<0.05. Significant difference *vs.* CSA group: [a]P<0.05.
Significant difference *vs.* CSA +SD group: [b]P<0.05.

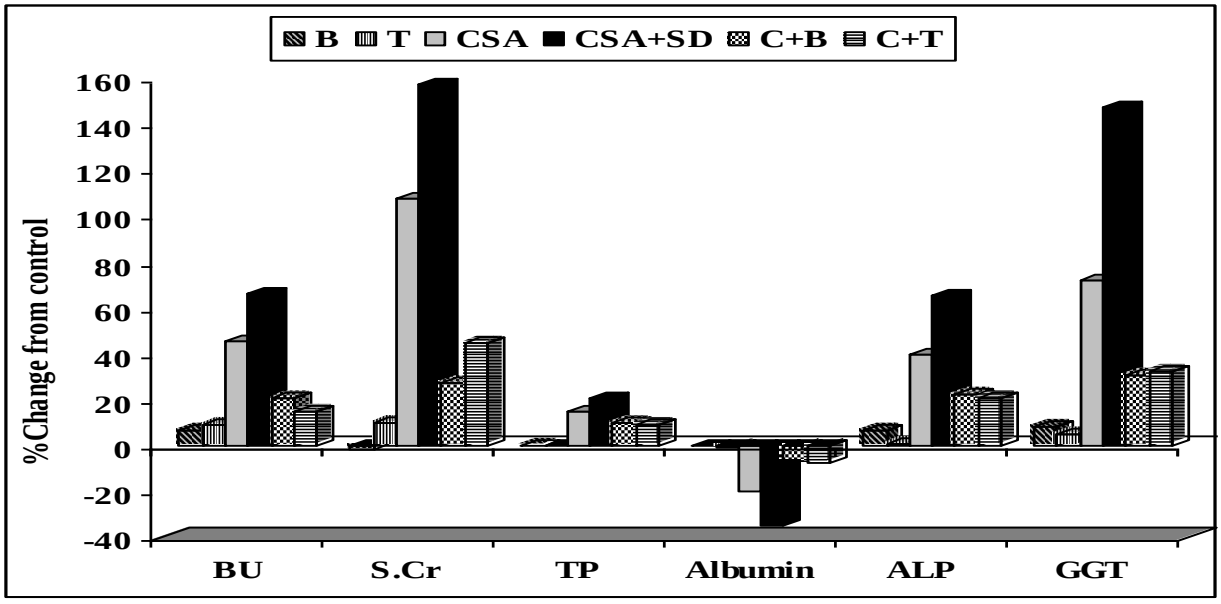

Fig. (1): % Change from control to show the effect of taurine and bismuth subnitrate on blood urea (mg/dl), serum creatinine (mg/dl), total protein (g/dl), Albumin (g/dl), alkaline phosphatase (U/L) and gamma glutamyltransferase (U/L) against cyclosporine and Sodium diclofenac -induced nephrotoxicity in ♀ rats after 6 weeks of treatment.

marked significant (P<0.05) increase in cell injury LDH release as well as significant (P<0.05) increase of the level of GSSG and reduction in the GSH/GSSG ratio (0.69% of control). These data suggest the role of oxidative stress in CSA nephrotoxicity. Treatment with CSA showed an increase in O^{-}_{2}, H_2O_2 and OH^{-} radicals production as described by Tariq *et al*. (1999) and Hagar (2004).

Lipid peroxidation begins as a result of oxygen derived free radicals (ODFR)-induced abstraction of hydrogen from a polyunsaturated fatty acid of cellular membrane forming a lipid radical, which is accompanied by cellular degeneration. Oxygen radicals are considered as important modulators of renal blood flow and glomerular filtration rate (Tariq *et al.,* 1999 and Capasso *et al.,* 2008). An efficient endogenous antioxidant defense system operates to compact free radicals. The main detoxifying system for lipid peroxides is GSH. The decrease in GSH following CSA observed in this study greatly supported this hypothesis (Tariq *et al.,* 1999 and Galletti *et al.*. 2005).

The effect of CSA on total protein, albumin, GGT and ALP

Accompanied to CSA- induced oxidative stress, a hepatotoxicity was implicated secondary to nephrotoxicity that was assessed by reduced serum total protein level and albumen, increased serum level of GGT and ALP, as depicted in table (1) and fig. (1). The present results are in agreement with the study of Hagar (2004). Also Briner *et al.* (2008) found that CSA induced transient rise in plasma ALP in kidney transplant patients.

The effect of CSA on kidney tissue

Confirming the biochemical results, the histopathological examination made on the kidney of CSA-treated rats, showed structural abnormalities in the kidney including swelling and degeneration in the epithelial cell lining the tubules (Fig. K2) and focal fibrosis in corticomedullary junction (Fig. K3). Atrophy was observed in some glomeruli while the others showed hypertrophy (Fig. K4) *vs* to the normal structure of CON (-ve control) and in T & B groups (+ve controls) (Fig. K1). Histopathological changes of kidney structure were recorded with earlier investigators who reported that CSA induced tubular vacuolation and necrosis, interstitial fibrosis (Tariq *et al.,* 1999 and Lim *et al.* 2004). Sanchez-pozos *et al.*, (2010) declared that CSA produced renal dysfunction and induced the development of arteriolopathy, TI-fibrosis and tubular apoptosis. Both acute and chronic administrations of CSA have been shown to increase renal vascular resistance (Mason 1990). Many substances such as angiotensin and nitric oxide are regarded as possible mediators of CSA-induced vasoconstriction (Tariq *et al.,* 1999). However, recent studies suggest an important role of endothelin in CSA-induced increase in vascular resistance (Bobadilla and Gamba, 2007). Endothelin has also been shown to affect rennin-angiotensin system and inhibit NO and prostaglandin production leading to vasoconstriction (Shihab *et al.,* 2003).

The effect of co-administration of CSA and SD on nephrotoxicity

Co-administration of CSA and SD increases the pain relieving efficacy in patients with rheumatoid arthritis. However, clinical studies showed that combined administration exaggerated cyclosporine nephrotoxicity. So, the first aim of this study is to visualize the possible extra nephrotoxicity by CSA in combined treatment.

Data in the tables (1,2) and figs. (1, 2) showed that SD induced an additive effect in all biochemical parameters of CSA-induced nephrotoxicity. The data recorded in the combined treated group (CSA+SD) showed significant (P<0.05) difference in both renal function and the oxidative stress parameters in comparison to CSA-treated group alone. Consistently, Kim *et al.* (1999) reported that administration of SD alone did not result in significant renal dysfunction, but combination of gentamicin, an inducer of nephrotoxicity, became deleterious to renal function.

On the other hand, it was observed that SD alone is a powerful nephrotoxicant and a strong oxidative stress (Hickey *et al.,* 2001). They added also that SD-induced nephrotoxicity may involve production of ROS leading to oxidative stress and massive genomic DNA fragmentation and apoptotic cell death. Secondary to oxidative stress an increment (p<0.05) in the levels of ALP, serum albumen, LDH release reported in combined treatment *vs* CSA alone were in agreement with the earlier studies of Masubuchi *et al.* (1998) and Okbi *et al.* (2002).

Confirming the biochemical results, histopathological studies made in kidney tissue of combined treatment showed severe structural changes in kidney more than observed in CSA alone, the combined group showed tubular necrobiosis in corticomedullary portion (Fig. K5), sever congestion, swelling and proliferation in the endothelial cells of glomerular tuft (Figs. K6&K7) in comparison to structural changes in kidney observed in CSA alone .In conform, marked histopathological changes were also recorded in the combined treatment with SD and gentamicin (a powerful nephrotoxicant) characterized by tubular atrophy, interstitial fibrosis and progressive renal impairment (Kim *et al.,* 1999; Yazawa *et al.* 2004). Inhibition of renal prostaglandin

Table (2): The effect of taurine and BSN on serum MDA (umol/ml), LDH (U/L), GSH (mmol/100 mL), GSSG (mmol/100ml) and GSH/GSSH ratio, against cyclosporine and Sodium diclofenac-induced nephrotoxicity in ♀ rats after 6 weeks of treatment.

Animal group		CON	B	T	CSA	CSA+SD	C+B	C+T
Tested Parameters	MDA	0.73 ±0.02	0.68 ±0.03	0.76 ±0.02	1.33 ±0.04*	1.62 ±0.02*[a]	1.13 ±0.06*[b]	0.97 ±0.05*[b]
	LDH	168.25 ±15.4	170.88 ±16.9	171.33 ±17.9	243.88 ±17.3*	335.88 ±18.7*	214.13 ±17.6*[b]	212.13 ±15.6*[b]
	GSSG	2.74 ±0.07	2.76 ±0.06	2.75 ±0.03	3.40 ±0.08*	4.60 ±0.05*[a]	2.95 ±0.06*[b]	2.88 ±0.04*[b]
	GSH	85.50 ± 3.42	84.18 ±2.20	85.87 ±2.04	73.87 ± 4.41*	64.99 ±2.41*[a]	77.01 ±4.54[b]	79.18 ± 2.77[b]
	GSH/GSSH	31.2 ± 1.3	30.5 ± 1.6	31.2 ± 1.2	21.7 ± 2.1*	14.13 ± 2.1*[a]	26.1 ± 2.5[b]	27.5 ± 1.4[b]

Significant difference vs. CON group: *P <0.05. Significant difference vs. CSA group: [a]P <0.05.
Significant difference vs. CSA +SD group: [b]P <0.05.

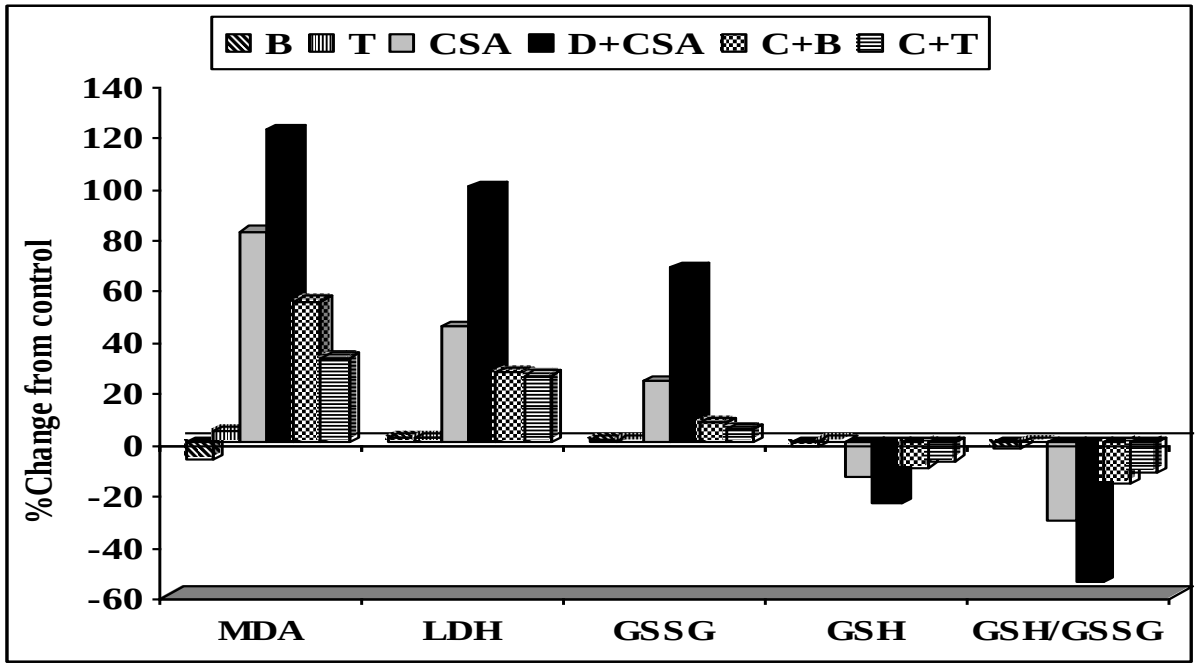

Fig. (2): % Change from control to show the effect of taurine and BSN on serum MDA (umol/ml), LDH (U/L), GSH (mmol/100 mL), GSSG (mmol/100ml) and GSH/GSSH ratio, against cyclosporine and Sodium diclofenac-induced nephrotoxicity in ♀ rats after 6 weeks of treatment.

synthesis by SD can lead to renal dysfunction which influence cortical blood flow, glomerular filtration rate and salt and water excretion (Griffin *et al.,* 2000). Also inhibition of cyclooxygenases after NSAIDs treatment may have a role in renal effects (Kim *et al.,* 1999). Considering this finding, the data strongly explored potential drug interactions when CSA co-administered with SD that cause deleterious effects to renal function and structure. The possible pharmacokinetic/dynamic study contributing to drug interactions reported in rheumatoid arthritis patients receiving CSA and NSAIDs, showed a negative relationship by CSA and SD co-administration and renal function (Mueller *et al.,* 1997). To interpret the latter observations it was mentioned that CSA induced nephrotoxicity through decreasing intrarenal PGE2 production mainly by decreasing COX2 expressing, i.e. minims the effect produced by SD. For that a prescription of NSAIDs, even COX2 inhibitor, should be very cautious in patients taking CSA (Chang *et al.* 2005). The data have strongly revealed that ROS mediate CSA-induced nephrotoxicity through increasing vascular resistance by inhibition of PGE2, leading to vasoconstriction and promotes fibrotic process that is characterized by tubular atrophy, interstitial fibrosis and progressive renal impairment (Shihab *et al.* 2003; Bobadilla and Gamba. 2007).

The 2nd target of this study was to investigate and compare the possible protective effects of taurine and BSN against nephrotoxicity induced by concurrent administration of CSA and SD. Concomitant oral administration of rats with either Tau or BSN attenuated the CSA and SD induced structure and functional changes in kidney.

As shown in table (1) and fig. (1) a significant ($P<0.05$) reduction in BU, S.Cr, serum ALP, GGT, TP and albumin under Tau and BSN treatments. In addition, the treatment antagonized deleterious effects of CSA and SD on oxidative stress markers, where it significantly ($P<0.05$) reduced the levels of serum MDA, serum LDH release and significantly ($P<0.05$) increase the level of serum GSH and GSH/GSSG ratio.

Protective effect of taurine

Reinforcing the biochemical results, histopathological examination of the kidney tissues of rats treated with taurine showed marked attenuation in all the CSA + SD-induced structural changes in kidney except some congestion in the cortical blood vessels (Fig. K8). In agreement, it was found that taurine administration (1% in the drinking water) reduced deteriorated renal function induced by CSA, as assessed by decreased serum creatinine, proteinurea levels and ameliorated CSA-induced morphological changes (Hagar *et al.,* 2006). The same authors also indicated that taurine in the same dose could decrease GGT level, increase serum TP, decrease hepatic MDA and increase level of GSH against CSA-induced hepatotoxicity. Taurine has been shown to decrease vascular resistance. It increased serum levels of nitric oxide and nitric oxide synthesis (Fennessy *et al.* 2003 and Hager *et al.* 2006), interfered with the activity of the renin-angiotensin-aldosterone system and minimized the elevation in serum cytokine, endothelin, thromboxane B_2 taurine also reduced oxygen derived free radical generation, up regulated the antioxidant defenses and inhibited the proliferation of vascular smooth muscle cells (Hu *et al.,*2009) which all lead to inhibit vasoconstriction and thus may decrease CSA-induced vascular resistance. Furthermore it was found that taurine can inhibit the hypoxia-induced expression of Entrolikin-1 mRNA and reduce the release of entrolikuin1and angiotensin II (Yu *et al.* 1997) that all may be contributing to ameliorate CSA-induced vascular resistance. CSA-induced nephrotoxicity is associated with accumulation of cellular calcium (Croft *et al.,* 1997), Taurine also can modulate calcium transport and has sulfhydryl group that have been shown to block calcium channels and maintain calcium homeostasis (Ruggenenti *et al.,* 1993). On the other hand taurine is a potent antioxidant and may attenuate tissue lipid peroxication either by scavenging a wide variety of ODFR including $O^{\cdot-}_2$, H_2O_2 and $OH^{\cdot\ -}$ radicals or by binding Fe^{2+} like a chelator, with HOCl and HOCl-metalloproteins, or by binding to or complexing the sulfonic acid group (SO_3-) to free metal ion species such as Fe^{2+}, Cu^{+} or oxidant metalloprotein (Erdem *et al.,* 2000). Treatment of rats with taurine attenuated CSA-induced depletion of GSH. GSH-dependent mechanisms play a vital role in protection of cells against oxidative stress and detoxification of xenobiotes including CSA (Inselman *et al.,* 1994). The major disadvantage is that GSH does not pass through the cell membrane and its action may be a function of its extracellular level only, on the other hand taurine readily passes through the cell membrane thereby resulting in quick replenishment of intracellular GSH. Finally, the data presented here suggest that concomitant use of antioxidant such as taurine might be useful in reducing CSA-mediated nephrotoxicity.

Protective effect of BSN

BSN is used as an antigastric ulcer and antidiarrhitic agent. It is suitable for inducing metalothionine (MT) in the kidney in cancer patients (Kondo *et al.,* 2004). However, due to the low absorption rate of Bismuth (Bi) from the

gastrointestinal tract, we used citrate as a vehicle for oral administration of BSN that increase the tissue distribution of Bi and enhance induction of MT in the kidney (Kondo *et al.,* 2004). MT is known to reduce the toxic effects of heavy metals, alkylating agents, inducers of ROS and γ- irradiation by virtue of its high content of sulfhydryl groups. Since CSA is an ROS inducer, the toxic effect of CSA could be greatly affected by MT and can be prevented by treatment with BSN. In agreement, it was shown that renal toxicity by CDDP can be prevented by MT induction in the target organ by administration of BSN in mice that reduced the elevated level of BU caused by CFFP treatment (Kondo *et al.* 2004). Moreover, BSN has been demonstrated to reduce cisplatin-induced renal cell death in clinical setting and during *in vivo* and *in vitro* animal experiment (Baelde *et al.,* 2003). Confirming the previous studies, the histopatological study on kidney of BSN-treated group showed mild improvement in kidney structure changes, where focal mononuclear leucocytes inflammatory cells infiltration was observed inbetween the tubules and congested blood vessels (Fig. K9).

The results demonstrated that bismuth subnitrate has a dual benefit effect as an ulcer-protection drugs and reno protective agent, which are considered the most common side effects for patients used to Co-administrate CSA and SD for long term treatment. It was found that BSN can bind and induce MT, as seen by the extended X-ray absorption fine structure spectrum of Bi_7MT is very similar to that for the glutathione and N-acetyl-L-Cystein complexes MT $[Bi(GS_3)]$ and $[Bi(NAC)_3]$ (Sun *et al.,* 1999). To interpret the latter observations, the result showed that BSN markedly increase the lower level of GSH induced by CSA. GSH is an efficient endogenous antioxidant defense system operates to compact free radicals and plays a vital role in protection of cells against oxidative stress and detoxification of xenobiotes including CSA. Thus induction of renal MT and GSH by BSN might be the major role of BSN in protecting renal function and tissue in CSA-induced oxidative damage and nephrotoxicity.

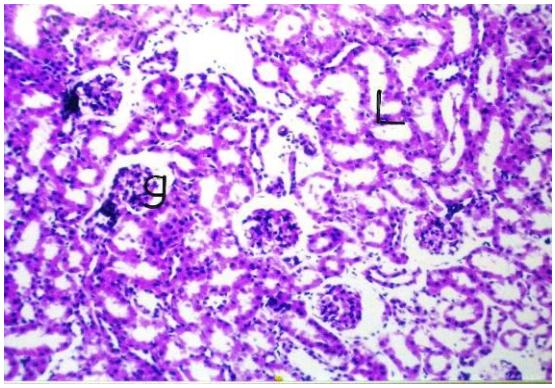

Fig . (K1) Kidney section of CON gp, showing the normal histological structure of the gloeruli (g) and tubules (L) in the cortex.

(H&E X 40)

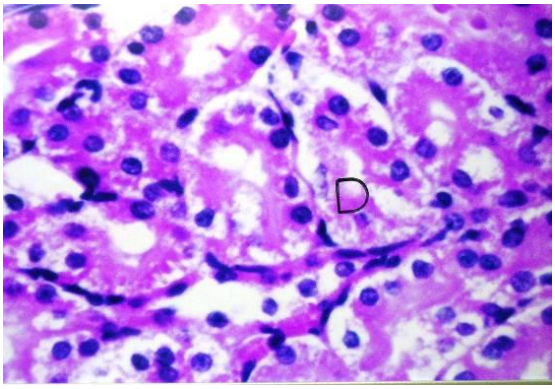

Fig. (K.2) Kidney of CSA gp, showing swelling and degeneration in the epithelial cells lining the tubules (D).

(H&E X 160)

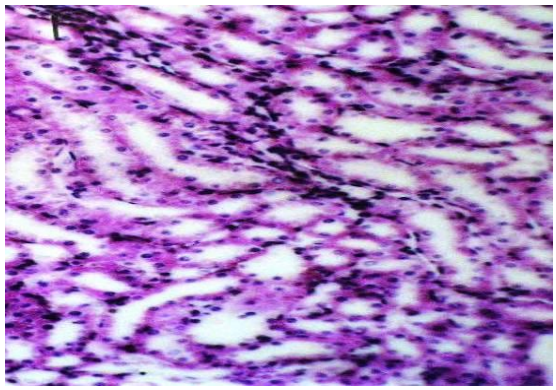

Fig . (K3) Kidney section of CSA gp, showing focal fibrosis in cortico-medullary junction (f).

(H&E X 160)

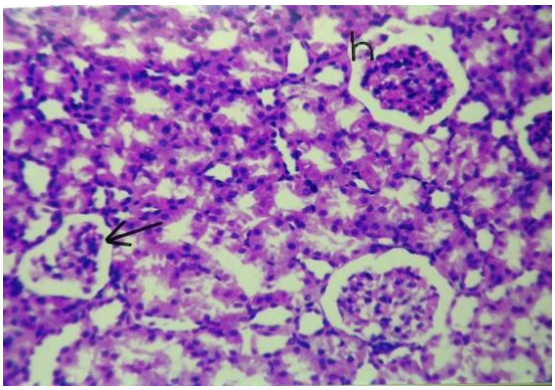

Fig. (K4) Kidney of CSA gp, showing atrophy in some glomeruli (arrow) and hypertrophy in others (h).

(H&E X 64)

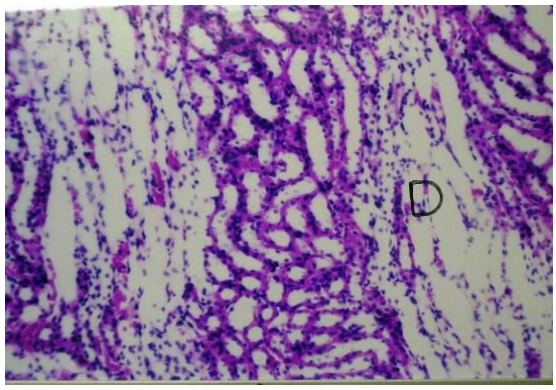

Fig. (K5) Kidney of CSA+SD gp, showing necrobiosis in the tubules at the corticomedullary (D).
(H&E X 40)

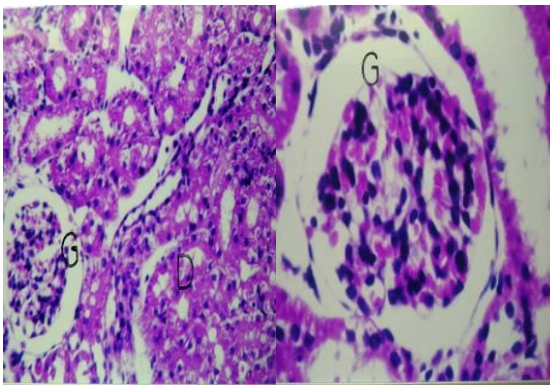

Fig (6K&7K) Kidney of CSA+SD gp, showing sever congestion of glomerular tuft (G) with degeneration and swelling in the lining epithelium of the renal tubules (D).
(H&E X64) & (H&E X160)

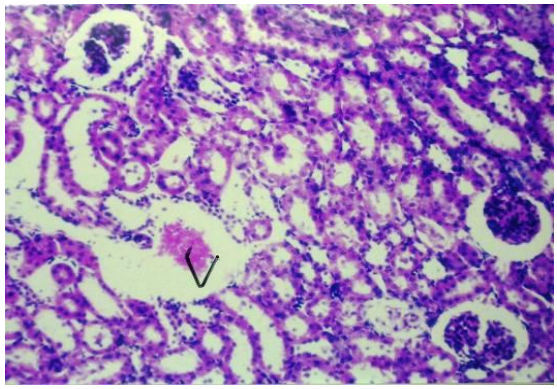

Fig. (K8) Kidney of C+T gp, showing congestion in cortical blood vessels (V).
(H&E X 40)

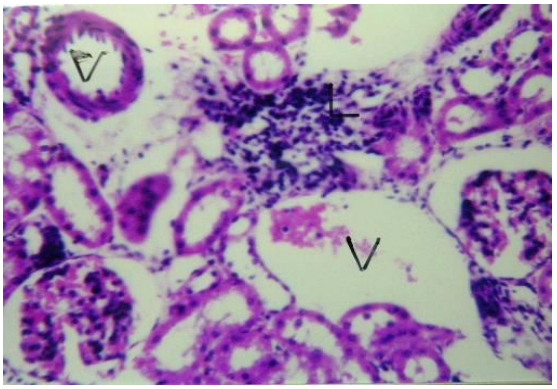

Fig. (K9) Kidney of C+B gp, showing congestion in the glomerular tuft (G) focal inflammatory cells infiltration (L) in between the tubules and congested and dilated blood vessels (V)
(H&E X 40)

4. Conclusion:

These results indicate that; 1) there is a negative relationship when CSA co-administered with SD and a positive one when the combined treatment co-administered with either tau or BSN. 2) ROS play the key role in mediating the negative effects of CSA. 3). Histopathological examination done on kidney sections reinforce the results obtained. 4) The potent effect of both Tau and BSN are somewhat similar but taurine appears more potent than BSN, in regaining the normal architecture of kidney tissue, and suggests a significant contribution of its antioxidant property to this benefit effect.

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Protective Effect of Spirulina Against Mitomycin C-Induced Genotoxic Damage in male Rats

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Abstract: *Spirulina platensis* (SP) is a filamentous cyanobacterium microalgae with potent dietary phyto-antioxidant, anti-inflammatory and anti-cancerous properties. The present study aimed to investigate the protective effect of Spirulina against Mitomycin C (MMC)-Induced genotoxic damage in male rats. To evaluate the protective role of *Spirulina platensis* expression alterations of the Bcl-2, CK8, CK19, p53, p21, and p27 genes and formation of micronucleus in male rats were investigated. Sixty Swiss albino male rats were divided into six groups. Group 1, animals were fed on a standard diet as untreated control group. Group 2 animals were fed on a standard diet mixed with 1% SP. Groups 3, animals were fed on a standard diet mixed with 1% SP powder followed by MMC (0.5 mg/kg). Group 4 animals were fed on a standard diet mixed with 1% SP powder followed by MMC (2 mg/kg). Groups 5 and 6 animals were fed on a standard diet followed by MMC (0.5 and 2 mg/kg, respectively. All the animals were sacrificed after an experimental period of 12 weeks. The expression of Bcl-2, CK8, CK19, p53, p21 and p27 genes was investigated using reverse transcription polymerase chain reaction (RT-PCR). The results revealed that MMC treatment induced expression alterations of genes related to apoptosis. Also MnPCEs formation was increased in bone marrow of male rats treated with MMC. These alterations of the gene expression as well as the MnPCEs formation were markedly suppressed when male rats were supplemented with SP for 12 weeks. Conclusion: These findings suggest that SP exerts its anti-mutagenic properties by inhibiting alterations in the gene expression and the MnPCEs formation in the hepatic tissues and bone marrow cells of male rats exposed to MMC.
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Keywords: *Spirulina platensis*, Mitomycin C, Gene expression; RT-PCR; Rats; MnPCEs formation.

Introduction

Mitomycin C (MMC) is a quinone-containing antibiotic originally isolated from Streptomyces caespitosus in 1958 (Wakaki et al.,). MMC has been used to treat a wide variety of solid tumors. Although current use of MMC is limited, this agent continues to be a key element in several clinical trials due to its intrinsic efficacy against many solid tumors and preferential activity in hypoxic tumoral cells Workman and Stratford (1993), MMC has a synergistic effect with radiotherapy via its radiosensitizing effects, targeting hypoxic cells in radiation resistant tumors, Sartorelli et al., (1994); Pors and Patterson(2005). To achieve its alkylating activity, MMC requires a bioreductive transformation to form active species that crosslink DNA, Dorr (1988); Na et al., (2001); Wang et al., (2007). Depending on the biotransformation pathway, metabolism of MMC may generate ROS Gustafson and Pritsos (1992). When ROS interact with cells and exceed endogenous antioxidant systems, there is indiscriminate damage to biological macromolecules such as nucleic acids, proteins, and lipids Offord et al., (2000).

Many plant-based chemopreventive agents are recognized to exert their anticarcinogenic effects by inhibiting cell proliferation and inducing cell differentiation and apoptosis. However, the chemo-preventive efficacies of these plants need to be tested in well established experimental animal tumour and genotoxic models, Subapriya et al., (2006) *Spirulina platensis* (SP) is a cyanobacterium being used in many countries as nutritional supplement for human and animal consumption. SP has been labelled as a powerful food, rich in proteins, carbohydrates, polyunsaturated fatty acids, sterols and some more vital elements like calcium, iron, zinc, magnesium, manganese and selenium. It is a natural source of vitamin B12, vitamin E, ascorbic acid, tocopherols and whole spectrum of natural mixed carotene and xanthophyll phytopigments Chamorro et al.,(1996); Piñero Estrada et al.,(2001); Chamorro et al.,(2002).

SP is well known for its anti-inflammatory and anti-cancerous properties. A hot water extract of SP has been orally administered to patients as an anti-

cancer and anti-viral agent. SP is best known as an immune booster by stimulating natural killer (NK) cells and co-operative action of IL-12 and IL-18 for NK-mediated IFN gamma production Hirahashi et al.,(2002).These SP-stimulated NK cells can fight illnesses other than cancer. SP hinders the growth of oral cancer. SP extract has been shown to inhibit tumor initiation in Syrian hamster cheek pouch mucosa painted with 7,12-dimethylbenz[a]anthracene Grawish (2008).Such an inhibitory effect may be attributed to the repair of carcinogen- damaged DNA, and SP has been suggested as an efficient radical scavenger, Romay et al.,(1998); Vadiraja et al.,(1998); Upasani et al.,(2001); Premkumar et al.,(2004).Other studies have reported that the unique polysaccharides of SP enhance cell nucleus enzyme activity and potentiate the process of DNA repair, Pang et al.,(1988); Kaji et al.,(2002).

Since cellular harm produced by MMC is thought to be at least partially due to a free radical mechanism, and MMC generates micronuclei-induced genotoxic damage in animal models, Hayashi et al.,(1992); Grisolia et al.,(2002) ,the aim of this work was to assess the genotoxic effect of MMC. These effects were measured as the number of micronucleated polychromatic erythrocytes (MN-PCE) from the bone marrow cells and the ability of MMC to induce alterations in gene expression of several genes related to cell apoptosis (Bcl-2, CK8, CK19, p53, p21 and p27) in hepatic tissues. We also assessed the potential protective action of SP against both micronuclei formation and changes in the gene expression due to MMC.

2. Materials and Methods

2.1. Materials:

2.1.1. Chemicals

Reagents and solvents used in the current study were of the highest possible grade available. The Mitomycin C was purchased from Sigma-Aldrich (USA). Reagents for RT-PCR method were purchased from Invitrogen (UK) and Fermentas (Germany).

2.1.2. Experimental Animals

Sixty Swiss albino male rats weighing 80-100 g were obtained from the Animal House at King Fahad Medical Research Centre, King Abdul Aziz University, Saudi Arabia. The animals were kept individually in wire bottomed cages at room temperature (25 ± 2 ºC) under 12 h dark-light cycle. They were maintained on standard laboratory diet and water *ad libitum*. The animals were allowed to acclimatize their new conditions for one week before commencing experiment, then they were distributed into eight groups (10 rats/ group). All animals received human care in compliance with the guidelines of the Ethical Committee of Medical Research, King Abdul Aziz University, Saudi Arabia.

2.1.3. Preparation of SP extract

SP algae used in this work was purchased from local market in Saudi Arabia. Then SP was cultured in our laboratory at King Abdul Aziz University, under optimal conditions on Zarrouk medium Andrade and Costa (2008). Algal mass was harvested every 3 weeks by continuous centrifuge, air dried and ground to powder form.

2.2. Methods:

2.2.1. Experimental design

After an acclimation period of one week, male albino rats 60-day-old (n=10 per group) were treated for 12 weeks and divided into the following groups: Group 1 (Untreated control group): animals were fed on a standard diet and given water *ad. libitum* for 12 weeks; Group 2 (SP-treated group): animals were fed on a standard diet mixed with 1% SP powder, and given water *ad. libtium* for 12 weeks; Group 3 (SP-MMC_1-treated group): animals were fed on a standard diet mixed with 1% SP powder, and given water *ad. libtium* for 12 weeks followed by MMC (0.5 mg/kg) dissolved in saline and injected intraperitoneally in a single dose 24h prior to sacrifice; Group 4 (SP-MMC_2-treated group): animals were fed on a standard diet mixed with 1% SP powder, and given water *ad. libtium* for 12 weeks followed by MMC (2 mg/kg) dissolved in saline and injected intraperitoneally in a single dose 24h prior to sacrifice; Group 5 (MMC_1-treated group): animals were fed on a standard diet and given water *ad. libtium* for 12 weeks followed by MMC (0.5 mg/kg) dissolved in saline and injected intraperitoneally in a single dose 24h prior to sacrifice; Group 6 (MMC_2-treated group): animals were fed on a standard diet and given water *ad. libtium* for 12 weeks followed by MMC (2 mg/kg) dissolved in saline and injected intraperitoneally in a single dose 24h prior to sacrifice.

During treatment, animals were observed twice daily for signs of moribundity and mortality. Body weights were recorded initially, once weekly, and at termination. At the end of the experimental period, the animals were rapidly sacrificed and the samples of the liver tissues and bone marrow cells of each animal were taken for gene expression and micronucleus analyses, respectively. Liver tissues were snap-frozen in liquid nitrogen and were kept at -80°C until analysis

2.2.2. Micronucleus test

The bone marrow cells resuspended in a small volume of fetal calf serum on a glass slide were used for smear preparation. The smear of bone marrow cells was prepared from each rat. After air-drying, the slide was fixed in methyl alcohol for 10 min and stained with 5% Giemsa stain for 10 min. Three slides were prepared for each animal and were coded before observation and one was selected for scoring. From each coded slide, 3,000 polychromatic erythrocytes (PCEs) were scored for the presence or micronuclei under oil immersion at high power magnification. In addition, the percentage of micronucleated polychromatic erythrocytes (%MnPCEs) was calculated on the basis of the ratio of MnPCEs to PCEs, Adler (1984)

2.2.3. Semi-quantitative Reverse Transcription-PCR

2.2.3.1. RNA extraction

Stored liver tissue samples (at −80°C prior to extraction), were used to extract the total RNA. Total RNA was isolated from 100 mg of tissues by the standard TRIzol extraction method (Invitrogen, UK) and recovered in 100 µl molecular biology grade water. In order to remove any possible genomic DNA contamination, the total RNA samples were pre-treated using DNA-free™ DNase treatment and removal reagents kit (Ambion, Austin, TX, USA) following the manufacturer's protocol. The RNA concentration was determined by spectrophotometric absorption at 260 nm.

2.2.3.2. Synthesize of First-strand cDNA

To synthesize the first-strand cDNA, 5 µg of the complete Poly(A)$^+$ RNA isolated from rat samples was reverse transcribed into cDNA in a total volume of 20 µl using 1 µl oligo (poly(deoxythymidine)$_{18}$) primer, El-Makawy, et al;,(2008) . The composition of the reaction mixture consisted of 50 mM MgCl2, 10x reverse transcription (RT) buffer (50 mM KCl; 10 mM Tris-HCl; pH 8.3), 200 U/ µl reverse transcriptase (RNase H free), 10 mM of each dNTP, and 50 µM of oligo(dT) primer. The RT reaction was carried out at 25°C for 10 min, followed by 1 h at 42°C, and finished with denaturation step at 99°C for 5 min. Afterwards the reaction tubes containing RT preparations were flash-cooled in an ice chamber until being used for DNA amplification through polymerase chain reaction (PCR) Ali et al;,(2008).

2.2.3.3. RT-PCR assay

The first strand cDNA from different mammary tissue samples was used as templates for the semi-quantitative RT-PCR with a pair of specific primers in a 25-µl reaction volume. The sequences of specific primer and product sizes are listed in Table 1. β-Actin was used as a housekeeping gene for normalizing mRNA levels of the target genes. The reaction mixture for RT-PCR was consisted of 10 mM dNTP's, 50 mM MgCl2, 10x PCR buffer (50 mM KCl; 20 mM Tris-HCl; pH 8.3), 1U/ µl taq polymerase, and autoclaved water. The PCR cycling parameters of the studied genes (Bcl-2, CK8, CK19, p53, p21 and p27) were performed as the PCR condition summarized in Table 1. The PCR products were then loaded onto 2.0% agarose gel, with PCR products derived from β-actin of the different rat samples**.** Each reaction of the RT-PCR was repeated with ten rats, generating new cDNA products at least ten times per each group.

2.2.4. Statistical Analysis:

All data were analyzed using the General Liner Models (GLM) procedure of Statistical Analysis System, SAS, 1982. followed by Scheffé-test to assess significant differences between groups. The values are expressed as mean ± SEM. All statements of significant were based on probability of $P < 0.05$.

Table I. Primers and PCR thermocycling parameters

Primer	Sequence (5′-3′)	PCR conditions	RT-PCR (bp)
Bcl-2	GGT GCC ACC TGT GGT CCA CCT G CTT CAC TTG TGG CCC AGA TAG G	42°C for 1 h, 95°C for 15 min, 32 cycles of (i) 94°C for 30 s, (ii) 62°C for 30 min, (iii) 72°C for 1 min and 72°C for 10 min	376
Cytokeratin 8	TTCCTGGAGCAGCAGAACAA	25 cycles: 94°C, 30 s; 65°C, 30 s;	255

CK8	GAGG ACAAATTCGTTCTCCAT	68°C, 1 min Final extension: 68°C, 2 min	
Cytokeratin 19 CK19	A TTCTTGG TGCCACCATTGA TCCTCATGGTTCTTC TTCAGG	30 cycles: 94°C, 30 s; 65°C, 30 s; 72°C, 1 min Final extension: 72°C, 2 min	238
p53	CGCAAAAGAAGAAGCCACTA TCCACTCTGGGCATCCTT	25 cycles: 94°C, 30 s; 65°C, 30 s; 68°C, 1 min Final extension: 68°C, 2 min	118
p21	ACCTCTCAGGGCCGAAAAC TAGGGCTTCCTCTTGGAGAA	25 cycles: 94°C, 30 s; 65°C, 30 s; 68°C, 1 min Final extension: 68°C, 2 min	88
p27	CAGAGGACACACACTTGGTAGA TCTTTTGTTTTGAGGAGAGGAA	35 cycles: 93°C, 30 s; 56°C, 45 s; 74°C, 45 s Final extension: 74°C, 10 min	124
β-Actin	GTGGGCCGCTCTAGGCACCAA CTCTTTGATGTCACGCACGATTTC	25 cycles: 94°C, 30 s; 65°C, 30 s; 68°C, 1 min Final extension: 68°C, 2 min	540

3. Results

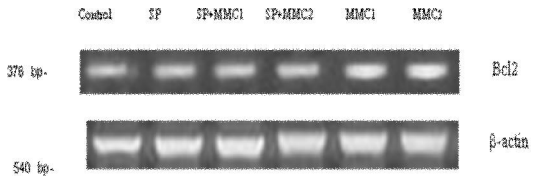
3.1. Rat survival and body weight

The results revealed that no significant differences in survival were observed between the untreated control, SP, and SP+MMC groups, with approximately 98% of the animals surviving to study termination (range = 92–99%). However, the survival rate between MMC animals was relatively decreased compared with control which reached 86%. The mean body weights of rats receiving SP or SP+MMC did not significantly differ from controls over time. However, the mean body weight of rats exposed to MMC at low (0.5 mg/kg) or high dose (2 mg/kg) was only 85% and 81% that of controls by the end of the study, respectively.

3.2. Semi-quantitative RT-PCR

Reverse transcription polymerase chain reaction was conducted to verify the expression levels of the Bcl-2, CK8, CK19, P53, P21, and P27 genes related to cell apoptosis in liver tissues of male rats (Table 1). Supplemented with SP for 12 weeks and exposed to several doses of MC 24h prior to sacrifice.

The results of the present study revealed that expression level of Bcl2 gene was significantly higher in hepatic tissues of MMC groups than control and other SP groups (Fig. 1). However, the expression level of Bcl2 gene in SP treatment was significantly lower compared with MMC groups and was similar to control group. Moreover, this expression level was significantly also lower in SP plus the low and high does of MMC groups than MMC groups. On the other hand, the Bcl2 expression in 0.5 mg/kg of MMC group was lower than the 2 mg/kg of MMC group (Fig. 1).



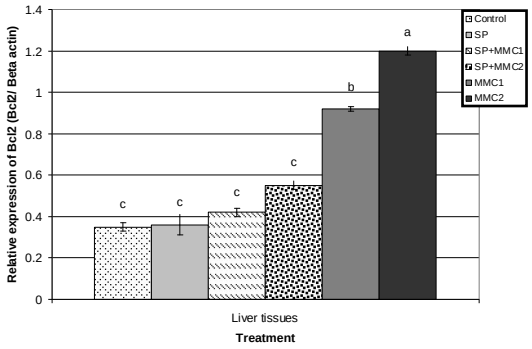


Figure 1: Semi-quantitative RT-PCR confirmation of Bcl2 gene in liver tissues of male rats treated with Spirulina (SP, for 12 weeks) with or without mitomycin C (MMC, at 24h prior to sacrifice) (a&b). Within each column means superscripts with different letters are significantly different (P ≤ 0.05).

The expression level of CK8 and CK19 genes in the hepatic tissues of male rats exposed to MMC at the low and high doses was significantly higher than control and other SP groups (Fig. 2 and 3). Moreover, the expression level of this gene in both MMC groups was relatively similar. However, these genes showed expression level significantly lower in SP plus MMC groups than MMC group alone (Fig. 2 and 3).

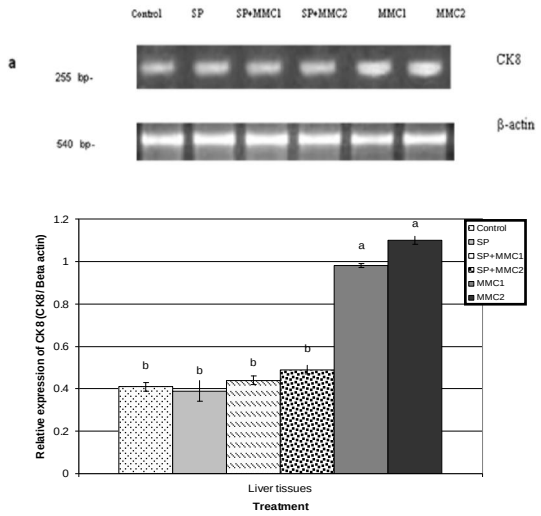


Figure 2: Semi-quantitative RT-PCR confirmation of CK8 gene in liver tissues of male rats treated with Spirulina (SP, for 12 weeks) and mitomycin C (MMC, at 24h prior to sacrifice) (a&b). Within each column means superscripts with different letters are significantly different (P ≤ 0.05).

From the determination of the expression level of gene p53 the results revealed that the expression level was significantly higher in hepatic tissue of MMC group than control and other SP groups (Fig. 4). However, the expression level of p53 gene in 2 mg/kg of MMC group was significantly higher than 0.5 mg/kg of MMC group. In contrast, this expression was significantly lower in SP plus 0.5 or 2 mg/kg of MMC groups than MMC groups (Fig. 4).

The expression level of p21 gene in the hepatic tissues of male rats exposed to MMC at the low and high doses was significantly higher than control and other groups (Fig. 5). However, the expression of p21 gene showed level of expression did not significantly change in SP plus 0.5 or SP plus 2 mg/kg of MMC compared with MMC groups. In the same trend, the p21 expression in SP alone was similar to control group (Fig.5).

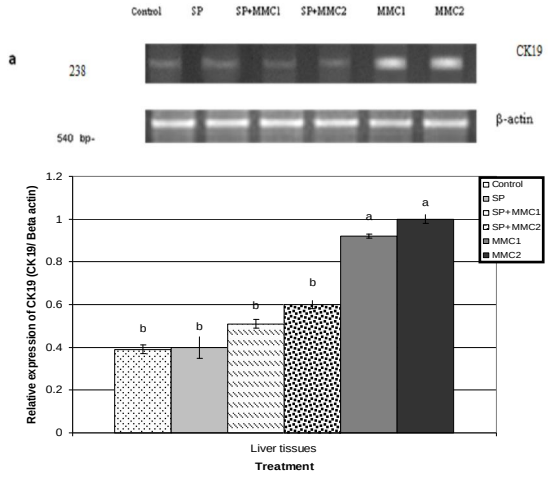


Figure 3: Semi-quantitative RT-PCR confirmation of CK19 gene in liver tissues of male rats treated with Spirulina (SP, for 12 weeks) and mitomycin C (MMC, at 24h prior to sacrifice) (a&b). Within each column means superscripts with different letters are significantly different (P ≤ 0.05)

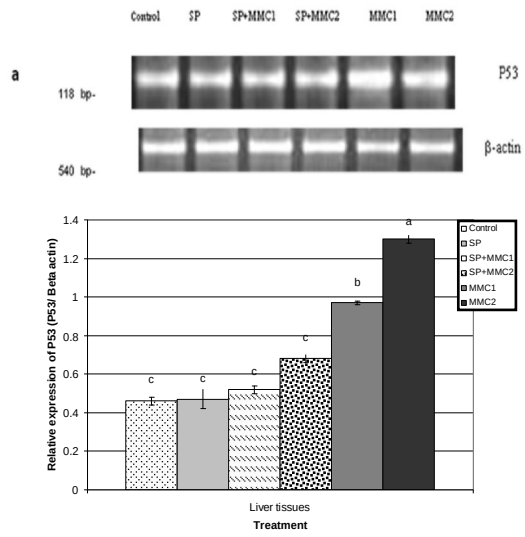


Figure 4: Semi-quantitative RT-PCR confirmation of P53 gene in liver tissues of male rats treated with Spirulina (SP, for 12 weeks) and mitomycin C (MMC, at 24h prior to sacrifice) (a&b). Within each column means superscripts with different letters are significantly different (P ≤ 0.05).

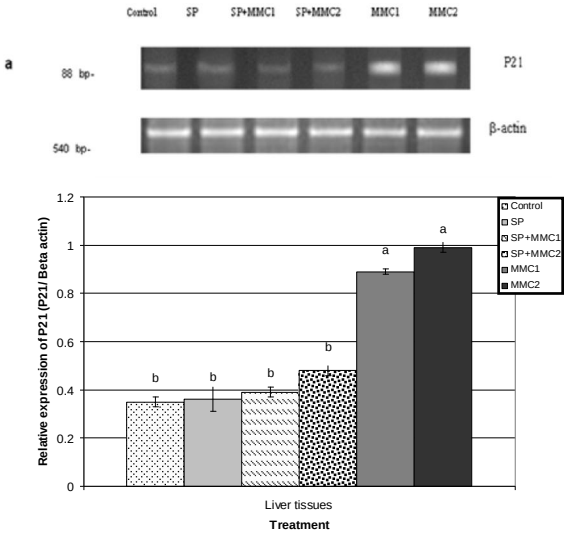


Figure 5: Semi-quantitative RT-PCR confirmation of P21 gene in liver tissues of male rats treated with Spirulina (SP, for 12 weeks) and mitomycin C (MMC, at 24h prior to sacrifice) (a&b). Within each column means superscripts with different letters are significantly different (P ≤ 0.05).

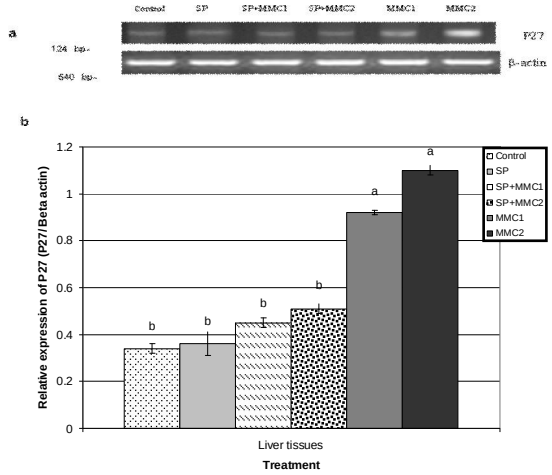


Figure 6: Semi-quantitative RT-PCR confirmation of P27 gene in liver tissues of male rats treated with Spirulina (SP, for 12 weeks) and mitomycin C (MMC, at 24h prior to sacrifice) (a&b). Within each column means superscripts with different letters are significantly different (P ≤ 0.05).

The expression profile of p27 gene was significantly higher in the hepatic tissues of male rats exposed to MMC at the low and high doses than control and other groups (Fig. 6). However, the expression level of p27 gene in SP plus all doses of MMC groups was not significantly different compared with control group. Also, this expression in SP alone was similar to control group (Fig. 6).

Table 2. Micronucleated polychromatic erythrocytes (MnPCEs) of male rats treated with Spirulina (SP, for 12 weeks) and mitomycin C (MMC, at 24h prior to sacrifice) (mean ± SEM).

Treatment	Conc. (mg/kg)	MnPCEs / 3000 PCEs
Control	---	8.1 ± 0.2^{c}
SP		8.9 ± 0.9^{c}
SP+MMC1*	1%+0.5	9.4 ± 0.8^{c}
SP+MMC2*	1%+2.0	10.2 ± 0.7^{c}
MMC1*	0.5	18.7 ± 1.1^{b}
MMC2*	2.0	24.3 ± 1.0^{a}

*: **Mitomycin C and MAA-QD were injected as a single dose and then the bone marrow cells were collected 24h after injection;** ^{a,b,c} **values with different superscripts within columns represent significant statistical differences (P< 0.05, Scheffé-Test).** ^{a,b,c} **values with similar superscripts within columns represent no significant statistical differences (P < 0.05, Scheffé-Test).**

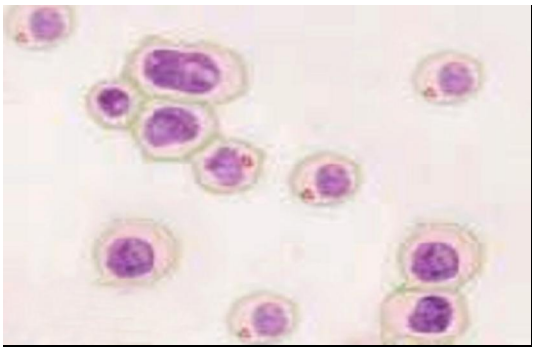


Fig. 7. Bone marrow erythrocytes of male rats treated with mitomycin C (MMC, at 24h prior to sacrifice). Arrow: micronucleated polychromatic erythrocyte (MnPCEs).

3.3. Micronucleus assay

Effect of SP and MMC on MnPCEs formation in the bone marrow cells of male rats is summarized in Table (2) and Figure (7). The results revealed that after SP supplementation for 12 weeks MnPCEs formation in the bone marrow cells was relatively similar to those in control group.

Treatment of male rats with 0.5 mg/kg of MMC at 24h before sacrifice increased significantly the formation of MnPCEs. Whereas, the formation of MnPCEs increased in the 0.5 mg/kg of MMC group to 230.7% compared to control group. In addition, MnPCEs formation in the 2 mg/kg of MMC group was significantly higher than all other group. Whereas, the formation of MnPCEs increased in the 2 mg/kg of MMC group to 300% compared to control group (Table 2).

On the other hand, exposure of male rats with SP plus low and high dose of MMC did not significantly increase the incidence of MnPCEs compared with control group. Whereas, the formation of MnPCEs in the bone marrow cells of these groups was relatively similar to control group (Table 2).

4. Discussion

The current work was carried out to investigate the effect of MMC on the expression alterations of the genes (Bcl-2, CK8, CK19, P53, P21 and P27) related to apoptosis and formation of Mn PCEs in male rats. Also the protective role of SP was determined to inhibit the alterations in the gene expression and suppress the Mn PCEs formation in male rats.

This study was associated with over-expression of the all tested genes in the MMC treated rats. These results were in great agreement of the results reported by Emi et al. (2005), who found that therapy treatment with doxorubicin (DOX), mitomycin C (MMC) on the human breast cancer cell line altered the expression level of several apoptosis genes. This was associated with over-expression of Bcl-2 and Bcl-xL genes in breast cancer cell line. Our results showed also over-expression of Bcl-2, p53, p21, p27, CK8 and CK19 genes in the liver tissues of male rats treated with MMC.

Bcl-2, a prominent anti-apoptotic member of the Bcl-2 family inhibits the release of pro-apoptotic molecules and cytochrome C from the mitochondria thereby inhibiting apoptosis and permitting persistence of tumour cells. In addition, over-expression of both p53 and Bcl-2 has been reported to inhibit transcriptional activation of Bax gene, Lucken-Ardjomande andMartinou(2005); Cavalier et al. (2005). Thus, over-expression of Bcl-2, p53, p21, p27, CK8, and CK19 genes in the liver tissues may confer a selective growth advantage on hepatic cells.

Administration of SP reduced the incidence of MMC induced rat hepatic tissues genetic alteration. Whereas, the SP was able significantly to down-regulate Bcl-2, p53, p21, p27, CK8 and CK19 expression in the hepatic cells at the dose of 1% of the rat diet. These results agreed the results of Ismail et al. (2009), who found that SP significantly down-regulated Bcl-2, PCNA and p53 expression in the liver of dibutyl nitrosamine (DBN)-treated rats.

The results of the present study substantiate the anti-genetic alteration properties of SP reported in literature. Polysaccharide extract from *Spirulina platensis* is a potent inhibitor of corneal neovascularization (CNV), decreased the expression of serine threonine kinase (AKT) and extracellular regulated kinase1/2 (ERK1/2) genes and that it may be of benefit in the therapy of corneal diseases involving neovascularization and inflammation Yang et al. (2009).

Roy et al. (2007) reported that reactive oxygen species (ROS)- levels determined in mouse macrophage cell line showed that C-Phycocyanin (C-PC), a biliprotein from Spirulina platensis was more effective in reduction of multidrug resistance (MDR1) gene expression. These results suggest that 2-acetylaminofluorene (2-AAF) induces MDR1 by ROS dependent pathway and C-PC is a potential a regulator of MDR1 expression.

To understand the regulation of the p53, p21 and p27 in the present study that it is known to induce apoptosis through inhibition of Bcl-2, amplification of death signals and activation of caspases, Haupt et al. (2003) . Mutations in p53 have been reported to occur in 40% of all human tumors. While wild type p53 functions as a TSG, mutant p53 functions as an oncogene. Mutant p53 protein is reported to have lost the ability to act as a growth suppressor and gained the ability to promote cell

proliferation. Over-expression of p53, p21 and p27 may enhance genetic instability by facilitating cell proliferation and inhibiting DNA repair and apoptosis. Furthermore, p53 activates telomerase and factors involved in angiogenesis as well as metastasis. In particular, p53 mutations have been reported to be associated with over-expression of Bcl-2, Haupt et al. (2003); Cavalier et al. (2005).

The present study clearly demonstrates that inhibition of cell proliferation and induction of differentiation and apoptosis may be major mechanisms through which SP exerts its anti-genotoxicity and anti-carcinogenic properties. This is the first report of the *in vivo* chemo-preventive effect of SP against MMC-induced rat liver genotoxicity and suggesting its potential use in chemoprevention of genetic alteration.

The investigation performed by Ismail et al. (2009), found that SP inhibited the incidence of liver carcinogenesis and prevented the expression levels of proliferating cell nuclear antigen (PCNA) and p53 were highly elevated in the liver of DBN-treated rats, but were significantly reduced by SP supplementation.

SP has been traditionally used for nutrition worldwide by people from Mexico, Africa and Asia. It is being widely studied for its possible antioxidant, antibacterial, and antiparasitic properties, and for several medical conditions such as allergies, ulcers, anaemia, heavy-metal poisoning, and radiation poisoning, Vadiraja et al.,(1998); Pors and Patterson(2005). SP or its extracts can prevent or inhibit cancer in humans and animals, Pang et al.,(1988); Schwartz and Shklar (1987); Mathew et al.,(1995); Qureshi et al.,(1996); Qureshi et al.,(1996);Chamorro et al.,(1996); Piñero Estrada et al.,(2001); Chamorro et al.,(2002); Kaji et al.,(2002).

In the present study, SP alone did not cause any side effects or organ toxicity, but it was remarkably effective in reducing the incidence of gene expression changes in the liver and MnPCEs formation in the bone marrow cells caused by MMC, suggesting its potential therapeutic effect in our model.

Mathew et al.,(1995) reported a chemopreventive role of SP against oral cancer. It has been suggested that the ability of SP to inhibit carcinogenesis is due to its anti- oxidant properties that protect tissues from cell damage, Khan et al. (2005). The potential hepatoprotective role of SP may be associated with its antioxidant constituents such as selenium, chlorophyll, carotene, gamma-linolenic acids, tocopherol, phenolic compounds content and vitamin E and C working individually or in synergy Kay (1991); Torres-Duran et al., (1999); Kaushik et al.,(2001); García-Martínez et al., (2007).SP has been shown to be effective against free radical induced cellular transformation, Romay et al.,(1998); Upasani et al.,(2001). In addition, phycocyanin, the main pigment present in SP, can inhibit cytochrome P450 mediated reactions involved in the formation of reactive metabolites of the hepatotoxins, Vadiraja et al.,(1998). Mittal et al. showed that SP significantly reduced the hepatic cytochrome P-450 content and significantly induced the hepatic glutathione S-transferase activity, Mittal et al.,(1999).

Moreover, Ismail et al. (2009), reported that *in vitro* studies revealed that polysaccharides of SP enhanced cell nucleus enzyme activity and DNA repair mechanisms, which are known to be closely associated with chemoprevention properties of natural products.

In the present study, liver and bone marrow cells of both control and SP did not express Bcl-2, p53, p21, p27, CK8, and CK19 genes and did not form the MN, respectively. However, liver sections of rats treated with MMC showed significant increase in the gene expression and MnPCEs formation, which was reduced by SP supplementation. This might be attributed to the anti-mutagenic effect of SP which minimized DNA damage caused by MMC.

Oxidative stress and chronic inflammation are closely associated with increased risk of cancer Wang et al.,(2002).. High concentrations of nitric oxide (NO) products generated by MMC can cause DNA damage, either directly or through secondary molecules, by nitroso- active deamination, DNA strand breakage, and DNA modifications Ambs et al.,(1997). NO-induced DNA damage can lead to p53 accumulation and p53-mediated apoptosis, Forrester et al.,(1996); Messmer and Brune (1996).

In summary, our study is the first to show that MMC-induced changes in the gene expression and MnPCEs formation in rat liver and bone marrow cells which were prevented by SP supplementation, suggesting that SP is a protective phyto-antioxidant against liver and bone marrow toxicity and an anti-genotoxic agent.

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Study of Sub-basal and Anterior Stromal Nerves of Corneal Flap with Modified Gold Chloride Stain

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Abstract: The aim was to study the regeneration of corneal nerve fibers following creation of corneal flap. **MATERIALS AND METHODS:** Nine white rabbits underwent creation of corneal flap only without the subsequent excimer laser photoablation, rabbits were scarified at 3 days, one week, two weeks and one month after the procedure. Demonstration of the corneal innervation was carried out with a modified gold chloride procedure. The tissue was dissected into 4-6 lamellae before dehydration and mounted on slides for observation and photography. **RESULTS:** At the 1st week, both superficial , basal epithelial and sub-epithelial nerves were found at the hinge of the flap but the rest of the flap showed a major loss of epithelial, basal subepithelial and superficial stromal nerves. At 1st month, A few new regenerating thin nerve fibres were found to emerge from the cut stromal nerve trunks. In addition,the anterior stromal nerve were thin with gradual restoration to its normal condition over time. At 6th month, The Sub-basal plexi returns to its pre-operative shape. The nerves of flap stroma become well developed. **CONCLUSION**: The number of sub-basal and stromal nerve fiber bundles almost completely disappeared after creation of flap. Sub-basal and anterior stromal nerves were still less than normal after 6 months.
***Key words*:** gold chloride, corneal nerves.

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Keywords: Sub-basal; Anterior; Stromal; Nerve; Corneal; Flap; Gold Chloride Stain

1. Introduction

The sensory innervation of the cornea is derived from the ophthalmic and maxillary branches of the trigeminal nerve. These nerve fibers are mylinated until they penetrate the limbus and form thick nerve bundles surrounded by the Schwann cells in the anterior third of the stroma ***(Zander and Weddell, 1951a).***

Each stromal bundle gave rise through repetitive branching to a moderately dense midstromal plexus and a dense subepithelial plexus (SEP). The SEP was comprised of modest numbers of straight and curvilinear nerves, most of which penetrated Bowman's membrane to supply the corneal epithelium, and a more abundant and anatomically complex population of tortuous, highly anastomotic nerves that remained largely confined in their distribution to the SEP. SEP density and anatomical complexity varied considerably among corneas and was less dense and patchier in the central cornea. A mean of 204 +/- 58.5 stromal nerves penetrated Bowman's membrane to supply the central 10 mm of corneal epithelium (2.60 nerves/mm^2). The density of Bowman's membrane penetrations was greater peripherally than centrally ***(Marfurt et al 2010).***

After entering the epithelium, stromal nerves branched into groups of up to twenty subbasal nerve fibers known as epithelial leashes. Leashes in the central and intermediate cornea anastomosed extensively to form a dense, continuous subbasal nerve plexus, while leashes in the peripheral cornea demonstrated fewer anastomoses and were less complex anatomically. Viewed in its entirety, the subbasal nerve plexus formed a gentle, whorl-like assemblage of long curvilinear subbasal fibers, 1.0-8.0 mm in length, that converged on an imaginary seam or gentle spiral (vortex) approximately 2.51 +/- 0.23 mm inferonasal to the corneal apex. Mean subbasal nerve fiber density near the corneal apex was 45.94 +/- 5.20 mm/mm^2 and mean sub-basal and interconnecting nerve fiber diameters in the same region were 1.51 +/- 0.74 microm and 0.69 +/- 0.26 microm, respectively ***(Marfurt et al 2010)***.

Intraepithelial terminals originated exclusively as branches of subbasal nerves and terminated in all epithelial layers. Nerve terminals in the wing and squamous cell layers were morphologically diverse and ranged in total length from 9 to 780 microm. The suprabasal layers of the central corneal epithelium contained approximately 605.8 terminals/mm ***(Marfurt et al 2010).***

In rabbits corneas the penetration of nerve bundles from the limbal area resembles that in human corneas. The rabbit corneas also exhibit stromal nerves that penetrate the uppermost a cellular stromal layer and gain access into the epithelium ***(Linna et al 1998).***

The gold chloride procedure was first used by Ranvier in late 1800's to visualize the structure of peripheral nerves. It was with this procedure that he observed the change in the continuity of myelin that he called the Node of Ranvier. This anatomically identified area of peripheral nerve is known by this name today. Ranvier's method of staining tissue whole mounts with gold chloride to visualize nerve fibers was modified by lengthening the incubation time in gold chloride and reducing the time in acidulated water. These simple modifications of an old technique give consistent impregnation of nerve fibers with light background staining in whole mounts of cornea and dura ***(Silverberg et al 1989).***

2. Materials and Methods:

2.1. Materials:

2.1.1. Experimental animals:

Nine white rabbits underwent creation of corneal flap only without the subsequent excimer laser photoablation.

2.1.2. Drugs:

Each animal received adequate anesthesia with intramuscular Ketamine (35 mg/ kg) and xylazine (5mg/kg) in addition to topical proparacaine HCL 0.5%.

2.2. Experimental design and methods:

Initially, nectatic membrane was cut before the procedure to prevent it from disturbing the flap. Eye lid speculum was placed between the lids and the eye was rinsed with 0.9% saline. A pararadial linear mark was dawn with a gentian violet pencil on the corneal surface. After placement of the suction ring, a superior hinge, 180µm, and 8.5 mm corneal flap was created, using Hansatome® microkeratome (B&L). Subsequently, the keratome was removed from the eye and the flap was lifted, using a cannula, rinsed well using 0.9% saline and repositioned without suturing. A suture was used to keep the lids closed for the first 24 hours. Antibiotic eye drops were administered 2 times daily for the first 3 days.

At the designated post-operative intervals, animals were sacrificed; the cornea was removed and stained with gold chloride.

Demonstration of the corneal innervation was carried out with a modified gold chloride procedure. We removed the cornea posterior to the limbus. We put the cornea into 5% formalin for 2 minutes. At the end of 2 minutes we drain off the solution and then section the cornea into 4 quadrants. With a sharp forceps we pick up each piece by the edge and put into the lemon juice for 10 minutes. We put each section of cornea into a bottle with about 5ml of 1% gold chloride. After 12 minutes we remove the cornea and put it in acidulated water for 16 hours. We remove the acidulated water and replace with 70% alcohol. The tissue was dissected into 4-6 lamellae before dehydration and mounting on slides for observation and photography.

3. Results:

3.1 Sub-basal nerves:

3.1. 1. After one week:

Nearly complete absence of the sub-basal nerve plexus in the basal cell layer was observed. (Figure 1).

3.1.2. After one month:

The starting regenerating sub basal nerves appeared short with few beads (Figure 2).

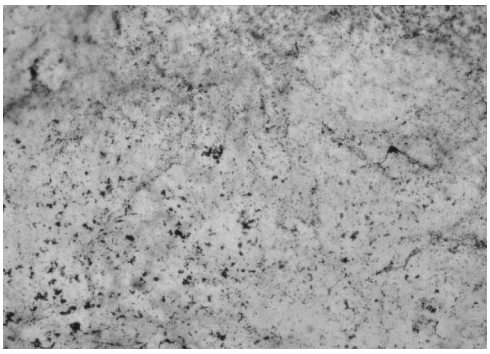

Figure 1: Complete absence of sub-basal nerve fibers at one week

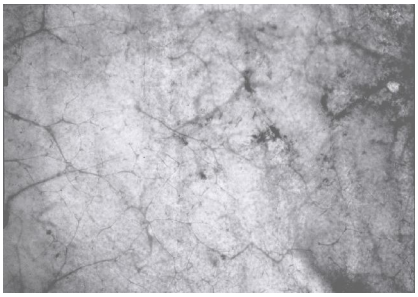

Figure 2: Starting regenerating sub basal nerves appeared short with few beads one month post surgery.

3.1.3. With further follow up:

The newly generated sub-basal nerves were slightly better constituted, especially in the centre of the flap. The Sub-basal plexus nearly returns to its pre-operative corneas in shape but not in density (Figure 3).

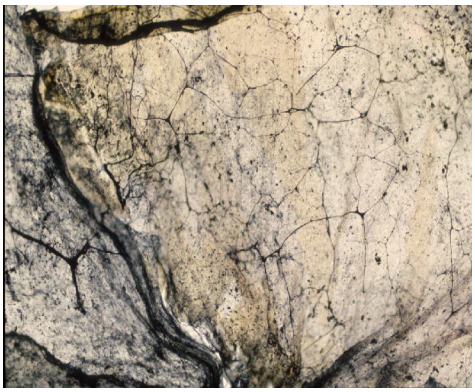

Figure 3: The Sub-basal plexus still less than its pre-operative shape at 6 month

Because most of the corneal stromal nerves lie within the anterior two thirds of the cornea, only the deepest stromal nerves avoid the microkeratome cut at the flap margin.

Only in the hinge area are spared as well developed epithelial and anterior stromal nerves are shown extending from the hinge to the flap (Figure 4).

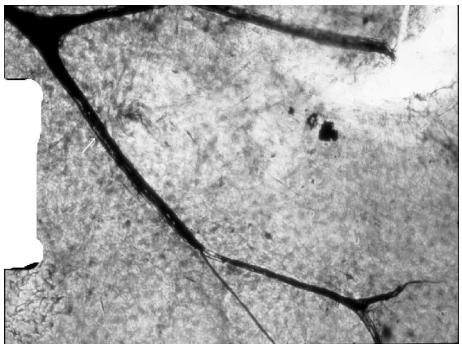

Figure 4: Superficial stromal nerves are seen at the hinge area

3.2. Aanterior stromal nerves:

3.2.1. At one week after the procedure

Some degenerated anterior stromal nerves in flap stroma were observed which appeared as very faint thin nerves (Figure 5).

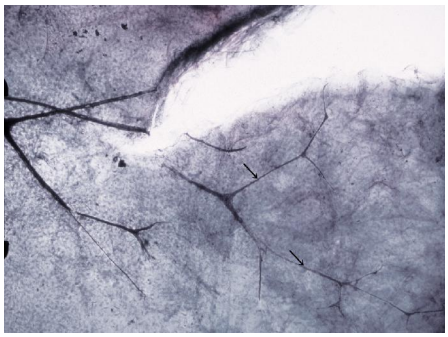

Figure 5: After superficial stromal nerves were cut at the nerve edges, the degenerated anterior stromal nerves were seen in flap stroma which appeared as very faint thin nerves (arrows)

3.2. 2. After one month of the procedure:

Numerous regenerating nerve fibers were observed to emerge from the cut stromal nerve trunks (Figure6).

Well-developed branched nerves start to appear in the anterior stroma of the corneal flap at six months.

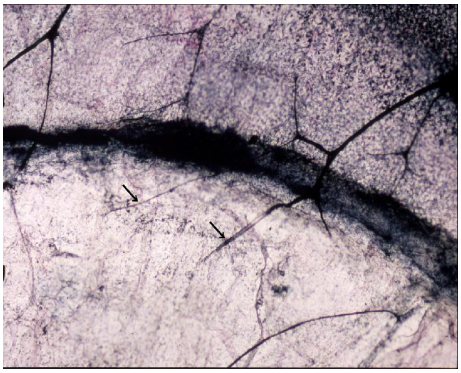

Figure 6: Thin single nerves started to appear in the flap stroma (arrows)

4. Discussion:

The rabbit and primate corneas shared relatively similar pattern of sensory innervation **(Rozsa et al 1983)** with some differences revealed by **(Muller et al 1996)**. While Bowman's layer is well developed in human corneas, this anterior a cellular stromal region is a less recognized structure in rabbits. The wound healing response of rabbit corneas is also considered to be more vigorous than in human.

The regeneration of stromal and epithelial innervations after LASIK has been studied using acetylcholinesterase histochemistry. Initially, in a rabbit study, only the hinge area was shown to preserve some of its stromal and epithelial innervation. In addition, occasional deep stromal nerve fibre bundles (NFBs) were observed to survive the micro-keratome cut under the flap. The cut stromal NFBs were found to send thin regenerating nerve fibers sometimes anastomosing with the neighboring stromal NFBs. These regenerating NFBs sometimes penetrated the most anterior a cellular stromal layer and sent subbasal NFBs forming the nerve terminals between the epithelial cells. By 2.5 months the anterior stromal, subbasal, and intraepithelial innervations was restored to near normal. The architecture of the deep stromal NFBs, however, remained abnormal even at 5 months. ***(Linna et al, 1998).***

Linna and co-workers, found that sub-basal nerve morphology seemed to degenerate from 1 week to 6 months after LASIK and corneal sensitivity returned to normal. All their patients had visible subepithelial nerve fiber bundles in their corneas. ***(Linna et al 2000).***

However, Lee and his associates reported significantly lower numbers of sub-basal nerve fiber bundles even 12 months after LASIK compared with the preoperative values with a superior hinge. Their findings were more consistent with the findings of Linna et al. The reason for the difference between results might be explained by the hinge position, since most nerves appear to enter the cornea at the nasal and temporal limbus. In their study, the regeneration of the nerves in the flap stroma was not complete up to 6 moths after LASIK, as reported earlier. They did not find any effect of LASIK on posterior stromal nerve fiber bundles, as expected ***(Lee et al 2002).***

In the sub-basal region, the number of nerve fiber bundles decreased by more than 90% 1 week after LASIK and was significantly lower at all times after surgery than it was before surgery. It increased 6 and 12 months after LASIK, but remained less than half of the preoperative value. In the stromal flap, the number of nerves at all times after surgery was also significantly less than before surgery and did not increase significantly by 1 year. In the stromal bed, there were no significant differences among any of the nerve measurements before and after LASIK ***(Lee et al 2002).***

It is found that corneal sub-basal nerve density does not recover to near preoperative densities until 5 years after LASIK, as compared with 2 years after PRK ***(Erie et al 2005).***

In other study, sub-basal nerve density decreased 82% in 5 days after LASIK. A gradual increase was observed from 2 weeks postoperatively, but even 2 years after the operation the nerve density was only 64% from the preoperative values ***(Moilanen et al 2008).*** Mean (SD) nerve density was decreased at 1 month compared with the preoperative examination and remained decreased through 12 months ***(Patel et al 2010).***

Corneal sub-basal nerve fiber density, nerve branch density, nerve fiber length, and nerve fiber width decreased significantly 1 month after LASIK and had not returned to the preoperative levels by 6 months. Nerve fiber tortuosity decreased significantly 1 month after LASEK and returned to the preoperative levels 3 months after surgery ***(Stachs et al 2010).***

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Simulation Optimization Approach for Facility Layout Problem-A Queuing Theory Based Approach

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Abstract: One of the most important issues in facility layout problem is to find the location of the Input/ Output points. We consider single loop path as material flow path for a given layout and find locations of Input/Out points on perimeter of the loop in the uncertain environment. The uncertainty is derived from production time of each department. Our objective is to minimize total time of AGV system after conveying all departmental material flows, we solve an uncertain queuing problem and due to difficulty of the queuing problem, an efficient simulation optimization approach is proposed using simulated annealing algorithm.
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Keywords: Facility layout; input/ output points location; queuing theory; simulated annealing

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 (Tompkins et al., 2003). Determining the most efficient arrangement of physical departments within a facility is defined as a facility layout problem (FLP) (Garey and Johnson, 1979). Tompkins (1997) stated that 8% of the United States gross national product (GNP) has been spent on new facilities annually since 1955. Layout problems are known to be complex and are generally NP-Hard (Garey and Johnson, 1979).

If the building size and shape are given, then the three principal and interdependent design decisions in the facility layout design problem are: (1) the determination of the shapes and locations of departments within the facility, which is called the conceptual block layout problem; (2) the determination of the locations of the input and output (I/O) points on the perimeter of each department; and (3) the design of the material flow paths or aisles that connect these I/O points (Kim and Goetschalckx, 2005).

In a basic layout design, each cell is represented by a rectilinear, but not necessarily convex polygon. The set of fully packed adjacent polygons is known as a block layout (Asef-Vaziri and Laporte, 2005). The two most general mechanisms in the literature for constructing such layouts are the flexible bay and the slicing tree (Arapoglu et al., 2001). A slicing structure can be represented by a binary tree whose leaves denote modules, and internal nodes specify horizontal or vertical cut lines (Wu et al., 2003). The bay-structured layout is a continuous layout representation allowing the departments to be located only in parallel bays with varying widths. The width of each bay depends on the total area of the departments in the bay (Konak et al., 2006).

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. We focus on single flow path AGV, one of the four well-known general types of design used in production systems (Apple, 1977). It lends itself to both product and production simplicity (Afentakis, 1989).

In determination of I/O points location, there are many research in the literature of facility layout problem. For example, Arapoglu et al., (2001) develop three constructive heuristics, a genetic algorithm (GA) and a simulated annealing (SA) algorithm to find location of I/O points. One of their three heuristic algorithms was deterministic and the other improved the first heuristic using uncertain parameters. They compared results of GA and SA algorithm with those of relaxed formulation and heuristics methods. Norman et al. (2001) and Kim and Goetschalckx (2005) integrate design of block layout and I/O points location problem. Norman et al. developed a heuristic algorithm to find I/O points location embedded in a GA algorithm. GA algorithm determined facility layout problem using bay. Kim and Goetschalkcx (2005) develop a SA algorithm to complete partial solution of layout developed by mixed integer programming (MIP) formulation and three heuristics are embedded in SA algorithm to find I/O points location. Ardestani Jaafari et al. (2010) consider I/O point location problem considering time value of money. They propose an MIP formulation to solve the problem considering I/O

stations with different capacity and costs of installment and maintenance.

Conventional approach for encountering with facility design problem was tended to consider all parameters in deterministic environment. This assumption is not appropriate in real world problems. There are two types of uncertain facility layout problem: material flows change in each period and they are deterministic in each period (dynamic layout) and in the latter, material flows change as random variables with known parameters in a single period (stochastic layout). Kulturel-Konak (2007) reviewed the importance of the uncertainty in the future of the facility layout problem.

One of the most important parameters in the facility layout problems is the production time of each department. When an AGV is used for transporting system, material flows have to wait for arriving an empty vehicle due to low capacity of vehicle. Using more than one AGV vehicle or vehicles with more capacity increase the cost of installment and maintenance of AGV systems. Transporting departmental material flows is a type of queuing problem. Finished goods and AGV vehicle are as customers and service provider respectively. There are a few researches in the literature of facility layout problem as the queuing problems.

Raman et al. (2009) discussed the development of a two step analytical approach to determine the quantity of material handling equipment that it is necessary for effective handling of products among facilities. At first, a solution is found by considering the time required for loading and unloading products, loaded travelling, empty travelling and breakdown of material handling equipment. Then a model is proposed to select the best alternatives between those generated from the first part in the stochastic nature. Jain et al. (2008) developed a queuing model for the prediction of flexible manufacturing systems performance using mean value. Smith (2009) presented some topological network design problems for material handling system design considering queuing network models.

In this paper, we discuss I/O points location problem as a queuing problem. Due to difficulty of the problem, we use simulation based optimization approach. A SA algorithm is developed to solve I/O point location problem in stochastic environment based on simulation optimization method. Each I/O point is as customer and AGV is service provider. Our objective is to minimize total time of traveling by AGV. We focus on single loop path; however our approach can be used for tandem. We also consider a single point I/O for each department and it can easily be extended to multiple I/O point with distinct point for input and output points of each department. Our approach can also be useful in routing problem when there multiple suppliers instead of a hub of supplier. The remainder of paper is organized as follows. Scetion 2 develops a SA algorithm. Section 3 gives computational results and efficiency of our approach in comparison with deterministic approach. Finally, section 4 concludes the paper and recommends some future studies.

2. SA Algorithm

To solve combinatorial optimization problems, simulated annealing algorithm is first proposed by Kirkpatrick et al. (1983). The name of SA algorithm is attained from the simulation of the annealing of solids. Annealing refers to a process of cooling material gradually to reach a steady state. In this process, a solid is heated until it melts, and then the temperature of the solid is slowly decreased (according to an annealing schedule) until the solid reaches the lowest energy state or the ground state (McKendall et al. 2006). SA algorithm is a well-established stochastic neighborhood search technique has a potential to solve complex combinatorial optimization problems (Gindy and Baykasoğlu 2001).

SA algorithm starts with a solution that is generated randomly. We represent an initial solution as a string that *i*th cell of the string shows the I/O point location of the *i*th department. We change location of I/O point of each randomly selected department. Enhancing moves are always accepted while no enhancing moves are accepted if

$$R = \exp\{-\Delta F / T\}$$

where R is a random number, ΔF is the increase in objective function and T is the current temperature. Temperature is decreased as follows:

$$T_{new} = \beta . T_{old}$$

We terminate SA algorithm when temperature is decreased to T_{end} . Since our input data are derived from the uncertain sources for each instance, we repeat running the SA algorithm to reach stable results.

3. Computational Results

It is important for any meta-heuristic algorithm to optimize their parameters, so we implement several experimental results to tune the parameters of the SA algorithm. The parameters are defined as follows:

T_0	Initial temperature
T_{end}	Final temperature
β	Cooling coefficient
M	Number of moves in each temperature

We consider a range of 100 to 150 for initial temperature, 0 to 10 for final temperature, 0.9 to 0.99 for cooling coefficient and 1000 to 1500 for the number of moves in each temperature. After running about 2000 randomly generated test problems with 10 types of size from 10 to 100, we set SA parameters as follows:

T_0	120
T_{end}	10
β	0.92
M	1200

We propose an approach for generating test problems as follows. We need to generate a block layout with it's shortest path single loop as well as probability density function, PDF, for production time of each department. We assume that AGV velocity is constant and equal to 1m per second. We consider production time of each department as N(μ_i, σ_i^2) that μ_i and σ_i^2 are randomly between (10,15) and (1,3) for each department respectively. We also generate matrix of material flow randomly between (1,10). AGV vehicle moves on the perimeter of the single loop path and loads the first finished goods. We consider single AGV vehicle with a unit capacity. When AGV is occupied, it is impossible to service other finished goods and material flow and they must wait for empty AGV. Moreover, location of I/O point is important in total time of the problem, because an I/O point can support more than one department at the same time. We generate 5 instances for 10 sizes ranging from 10 to 100 departments, merely 50 instances totally. We consider objective function as a probability variable labeled by X . Each instance is solved in several scenarios until the objective function is converged. For each instance, we have n scenario with x_i objective function (i=1,2,…, n). We show average of objective function of each scenario as follows.

$$\bar{x} = \sum_{i=1}^{n} x_i / n$$

We know that the difference between expected value of X and its' estimator $\bar{x}$ divided by standard deviation of $\bar{x}$ is a random variable with density function of t-student with parameter n, namely:

$$(E(X) - \bar{x}) / SD(\bar{x}) \sim t_{n-1}.$$

Using this equation, we find number scenarios for each instance equal to n.

Table 1 gives computational results. Objective function is total working time of AGV system and CPU time shows computational time to solve the problem in each instance. SA algorithm is coded in MATLAB software in a PC with 2.3 GHz Core2 Due CPU and 1GB RAM. In the first and second columns, size and number of each department are indicated respectively and in the third column, average total time of each size is shown. In Table 2, a comparison between the proposed approach and mean value approach is stated. We compare two approaches in four situations as follows:

1. Production time of each department is equal to μ_i
2. Production time of each department is equal to $\mu_i + \sigma_i^2$
3. Production time of each department is equal to $\mu_i - \sigma_i^2$
4. Production time of each department is a random number of Normal probability distribution N(μ_i, σ_i^2), Random Situation (RS).

We show efficiency of our proposed method respect to mean value method in different situations. We use a measure to show the effectiveness as follows:

$$(1 - Z_2 / Z_1) \times 100$$

where Z_1 is the objective function of mean value method and Z_2 is the objective function of our proposed method. Our proposed method is reasonably better than mean value method except situation 1, however, there is not any significant difference between two methods (less than 4% gap). In other 3 situations, especially in RS, there are a significant difference between two approaches with 13.7%, 13.6% and 16.3% in average for situations 2, 3 and 4 respectively.

4. Conclusions and recommendations

In this paper, we consider I/O point location problem with stochastic nature as a queuing model. We consider AGV vehicle as a single channel service provider and each department with uncertain production time. Computational results indicate flexibility of our proposed method in various situations. The proposed approach can be useful in many manufacturing systems. We recommend some extensions as follows. It can be useful to consider several AGV vehicles with multi capacity and investigate material handling cost and installment and maintenance costs. It is also recommended to present an approximation algorithm to estimate effectiveness of the proposed queuing model.

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Table 1. Computational results

Size	No	Objective	CPU Time	Size	No	Objective	CPU Time
	1	1096	22		26	2855	1211
	2	1022	16		27	2830	1256
10	3	1010	14	60	28	2988	1125
	4	1092	25		29	2942	1218
	5	1063	23		30	3044	1334
	6	1548	28		31	3423	2617
	7	1597	39		32	3220	2555
20	8	1592	28	70	33	3331	3654
	9	1596	28		34	3483	3720
	10	1514	27		35	3680	3649
	11	1736	158		36	3611	3379
	12	1728	159		37	3624	4434
30	13	1767	123	80	38	3534	3817
	14	1747	104		39	3769	2758
	15	1721	111		40	3770	3064
	16	2376	414		41	4578	3650
	17	2134	246		42	4241	4208
40	18	2214	340	90	43	4261	3579
	19	2274	426		44	4561	5218
	20	2107	311		45	4364	4226
	21	2519	682		46	4545	4867
	22	2541	782		47	4489	5720
50	23	2812	765	100	48	4775	6541
	24	2841	756		49	4611	7864
	25	2659	906		50	4252	7720

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Table 2. Comparing proposed approach with deterministic approach

Size	No	μ	μ+ σ	μ- σ	RS	Size	No	μ	μ+ σ	μ- σ	RS
	1	-0.2	7.3	7.3	9.9		26	-1.9	15.5	14.5	13.0
	2	-0.5	9.6	9.6	9.1		27	-1.8	15.1	21.5	12.2
10	3	-0.3	5.5	5.5	8.0	60	28	-0.6	17.2	21.4	19.2
	4	-0.2	9.0	9.0	8.6		29	-2.1	14.9	14.1	15.6
	5	-0.1	9.3	9.3	6.3		30	-1.4	21.2	20.7	19.9
	6	-0.9	14.4	15.1	8.7		31	-1.9	15.9	16.3	24.3
	7	-1.0	14.5	8.5	8.9		32	-2.7	13.3	11.1	20.4
20	8	-0.9	11.7	12.8	10.8	70	33	-0.5	14.5	11.1	11.7
	9	-1.4	11.5	8.4	9.1		34	-2.9	16.3	14.4	10.1
	10	-0.3	8.4	8.9	10.3		35	-1.9	14.7	14.1	22.8
	11	-0.2	10.4	16.1	14.5		36	-1.6	15.9	15.4	22.9
	12	-1.1	15.7	9.0	13.3		37	-1.1	15.7	12.2	19.2
30	13	-0.5	16.6	14.7	13.8	80	38	-0.9	11.1	11.2	18.7
	14	-1.9	12.5	10.4	14.8		39	-0.3	10.3	14.5	21.5
	15	-1.4	16.4	13.5	9.5		40	-0.5	11.2	10.9	22.0
	16	-0.16	15.2	15.9	23.7		41	-2.7	11.3	11.1	20.1
	17	-0.15	16.7	16.5	16.8		42	-3.7	16.1	16.8	17.2
40	18	-1.8	15.7	12.4	18.9	90	43	-2.1	13.5	12.3	20.8
	19	-0.27	13.9	21.1	20.1		44	-1.5	10.4	13.3	23.5
	20	-1.51	14.4	13.6	21.5		45	-1.4	10.7	15.2	20.2
	21	-2.9	15.3	20.9	22.6		46	-2.2	10.6	13.0	17.7
	22	-2.9	15.7	12.7	16.0		47	-2.5	12.7	16.1	22.5
50	23	-2.2	20.9	14.9	19.1	100	48	-3.3	10.3	12.8	15.3
	24	-1.4	21.8	18.7	10.0		49	-2.4	14.3	16.9	21.7
	25	-1.0	19.5	13.0	22.0		50	-3.0	12.2	13.2	16.8

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Sporicidal Effect of Ozone on Fungal and Bacterial Spores in Water Disinfection

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Abstract: The sporicidal effects of high ozone concentrations were tested against an endospore forming bacterial strain (*Bacillus subtilis* ATCC 6633) and a fungal strain (*Aspergillus brasiliensis* ATCC 16404) as a method of water disinfection. We compared the sporicidal action of ozone against these fungal and bacterial strains. Under identical treatment conditions, ozone showed a sporicidal effect on bacterial and fungal spores in water. Our present results showed that ozone concentrations at 7.0 and 9.0 g/m^3 have a sporicidal effect against bacterial and fungal spores respectively. Electron microscopic study of ozone-treated *B. subtilis* and *A. brasiliensis* spores mentioned above suggests the outer spore coat layers as a probable site of action of ozone. Our present study on ozone supports the notion that oxidizing agents including ozone probably kill spores by degrading the outer spore components and exposing the spore core to the action of the sanitizer. The ozone was generated using coaxial dielectric-barrier-discharge (DBD) technique. The coaxial DBD cell consists of two cylindrical coaxial electrodes separated by a gap distance and dielectric barrier (glass). AC (50 Hz) high voltage (2-5 kV) was applied on the DBD cell to generate filamentary discharge. The DBD cell is fed by oxygen gas. The basic mechanism of ozone generation simply consists of dissociation of oxygen molecules by the discharge electrons that are formed in the discharge filaments inside the discharge gap. The atomic oxygen, which is produced due to the dissociation, reacts with the oxygen molecules to form ozone. In the discharge, the oxygen molecules are dissociated prior to ozone formation. The concentration of the generated ozone was controlled by the discharge current and the gas flow rate. The generated ozone was used to treat the spores under investigation.
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Keywords: Sporicidal; Fungal; Bacterial; Ozone; water; Disinfection

1. Introduction

Water arrives in many ways: simple rain, floods, tornados, hurricanes, monsoons, snow, hail and sleet. These bring to organisms precious water needed for life. Without water, there would be no life. With water, there is life and among the living are molds, yeasts and bacteria. The molds, yeasts and bacteria can form spores. Spores are important for survival and dissemination (Donald, 2009).

Microbial life is abundant, tenacious, and is often very difficult to control. Organisms including viruses, bacteria, and fungi are often characterized by an ability to spread easily, reproduce rapidly, and thrive under conditions that can destroy higher life forms. Many of these organisms are completely harmless to humans and play an important role in many eco-systems and natural processes. Some organisms, though, cause human diseases, and exclusion or destruction of these organisms is important to prevent or block the spread of disease (Cross *et al*., 2003).

Bacterial spores are of considerable interest due to their remarkable resistance to physical agents and regular antiseptics. Their tolerance to heat and the fact that they can survive a number of years in a dried state are of great importance in medicine as well as in food preservation (Mathias *et al*., 2010). Microbiological spores are among the most resilient forms of dormant life known to man. Although formed by many different types of microbiological organisms, the most commonly studied spores are from various *Bacillus* or *Clostridium* species. In particular, *Bacillus* spores are amazingly resistant to common sterilizing techniques. For example, most vegetative bacteria die quickly when subjected to temperatures in excess of 80 ºC, but bacterial spores often survive boiling water at 100 ºC for two hours or more. Spores have survived for 20 years in 70% alcohol solutions. Drying has little effect on spores, as demonstrated by spores surviving in the intestines of Egyptian mummies for thousands of years (Alcamo, 2001).

Fungi are ubiquitous in nature (Goncalves *et al*., 2006). They are present in and have been recovered from a wide range of aquatic habitats including lakes, streams, distribution systems, drinking water and also on the surface of drinking water reservoirs and distribution pipes (Bitton, 2005). Fungi may cause some problems in drinking water. They are involved in the production of taste and odors in water (Bitton, 2005; Goncalves *et al*., 2006). Some of them form humic-like substances that may

act as precursors of trihalomethanes (Bitton, 2005). Health problems are also possible. Aspergillus is a group of filamentous fungi that account among the most frequently isolated fungi in water environments.

Aspergillus spp. could produce aflatoxins, a group of acutely toxic and potentially carcinogenic and immunosuppressive mold metabolites. Although aflatoxins are often associated with food but aflatoxins and *Aspergillus flavus* were detected from stored water (Goncalves *et al*., 2006). There are also evidences showing nosocomial aspergillosis, a life threatening infection in immunocompromised patients, that is thought to be primarily airborne may be waterborne in hospital environments (Anaissie and Costa, 2001; Anaissie *et al*., 2002). Waterborne Aspergillus species can aerosolize and their concentration is highest near water activity (Anaissie and Costa, 2001). Moreover fungal spores appear to be more resistant to chlorine and ozone than coliform bacteria and can remain viable for extended periods of time (Bitton, 2005).

An understanding of mold spores is important for control. Without control, mold spores can cause extensive damage of materials and goods. Mold spores are reproductive, they are like fertilized eggs; each spore can generate a new mold. One mold colony can produce millions of spores in a day. Mold spores are resistant to drying (desiccation) and heat, but they may be killed by boiling, ozone, and fungicides containing phenolics and quaternary ammonium treatments. Mold spore walls are chemically similar to their thread-like hyphae. However, spore walls are much thicker more than mold hyphae. The thick spore walls resist drying and protect against heat. Some molds like *Rhizopus* form spores inside a sporangium (Donald, 2009).

Ozone is a strong, fast and broad-spectrum antimicrobial agent that works effectively against bacteria, bacterial spores, viruses, fungi, fungal spores and protozoa. Unlike many other sterilizing agents ozone is easy and fast to remove after the process and does not leave any remaining chemicals, odor or taste. Inactivation of bacteria is thought to occur by ozone oxidizing the fatty acids in the cell-membrane and macromolecules, like proteins and DNA. The damage caused by ozone is irreversible and causes lysis of the cell wall and the death of the bacteria. It also kills spores and viruses as it oxidizes DNA and proteins in the spore as well as in viruses. The effect of ozone in water is well known and seems to be more effective than ozone in air. It has also been indications that ozone needs a higher humidity in the air to be really effective (Ronny, 2005).

Significant advantages of ozone in water are that it decomposes quickly to oxygen, leaving no residue of itself and few disinfection by-products, and it has more potency against bacteria, cysts of protozoa, viruses, and fungal spores than hypochlorite (White, 1999). Ozone can oxidize many organic compounds, particularly those with phenolic rings or unsaturated bonds in their structure (Razumovski and Zaikov, 1984), and can therefore have a role in reducing pesticide residues in process water (Nickols and Varas, 1992) and mycotoxins in durable commodities (McKenzie *et al*., 1997). Ozone attacks and dissolves the spore's coat, and an overly long exposure time eliminates any trace of the spore.

2. Material and Methods

2.1. The test microorganisms

2.1.1. Bacterial and fungal strains

Bacterial and fungal strains used in the course of this study were *Bacillus subtilis* ATCC 6633 and *Aspergillus brasiliensis* ATCC 16404. The strains were obtained kindly from the Department of Microbiology of Memphis Pharmaceutical Company, Cairo, Egypt. The bacterial strain was cultivated on Nutrient agar slants, while the fungal strain was cultivated on Malt extract agar slants.

2.1.1.1. Bacterial spores Preparation

To prepare *B. subtilis* ATCC 6633 spores (Lijie Li, 2004), a loopfull of *B. subtilis* was inoculated onto nutrient agar slants and incubated at 37°C for 10 days to sporulate. The spores were collected by rinsing the slants with sterile phosphate buffer and centrifuging at 4000 *rpm* for 4 minutes. Then the supernatant was withdrawn and washed twice with buffered water and centrifuged at 6000 *rpm* for 10 minutes. The final sporulated suspension was pasteurized in a 75°C water bath for 15 minutes with the purpose of killing all vegetative cells and to activate spore germination. The spore suspension was stored at 4°C for no more than one week until they were needed.

2.1.1.2. Cultivation of treated *Bacillus subtilis* ATCC 6633 spores

The treated spores were diluted, inoculated onto the surface of nutrient agar plates and incubated at 37°C for 24 hours (Lijie Li, 2004). At the end of incubation period, the number of colonies in the plates was counted (the number of colonies to be counted was between 20 and 80 colony/plate).

2.1.1.3 Determination of viable spores concentration (CFU/ml)

The viable spores (bacterial or fungal) concentration was computed and expressed (Lijie Li, 2004) as colony forming units (CFUs)/ml as shown in the following equation:

$$CFU/ml = \sum Ni / \sum Vi$$

Where Σ is the summation taken overall plates of the particular sample, Ni is the colonies counted in a plate, Vi is the actual volume (ml) of the sample in a plate.

2.1.1.4. *A. brasiliensis* ATCC 16404 spores Preparation

Fungal spore preparation was carried out as described by Mahnaz and Hossein (2008). Indigenous spores were prepared by streaking on malt extract agar medium. The pure culture was inoculated into 10 ml malt extract broth tubes and after 5 days incubation at 37°C, spores were collected and harvested by washing with sterile deionized water containing 1% Tween 80. Spores densities were determined microscopically by haemocytometer and controlled by spreading plate of 200µl serially diluted suspensions on malt extract agar. The suspension of concentrated spores was stored at 4°C until they were needed.

2.1.1.5. Cultivation of treated *A. brasiliensis* ATCC 16404 spores

The treated spores were diluted, inoculated onto the surface of malt extract agar plates and incubated at 37°C for 5 days. At the end of incubation period, the number of colonies in the plates was counted (the number of colonies to be counted was between 20 and 80 colony/plate).

2.2. Ozone generation

The ozone was generated using coaxial dielectric-barrier-discharge (DBD) technique. The coaxial DBD cell consists of two cylindrical coaxial electrodes separated by a gap distance and dielectric barrier (glass). AC (50 Hz) high voltage (2-5 kV) was applied on the DBD cell to generate filamentary discharge. The DBD cell is fed by oxygen gas. The basic mechanism of ozone generation simply consists of dissociation of oxygen molecules by the discharge electrons that are formed in the discharge filaments inside the discharge gape. The atomic oxygen, which is produced due to the dissociation, reacts with the oxygen molecules to form ozone. In the discharge, the oxygen molecules are dissociated prior to ozone formation. The concentration of the generated ozone was controlled by the discharge current and the gas flow rate was adjusted to 5 L/min (Garamoon *et al*., 2009).Ozone was applied directly into the tubes containing the bacterial and fungal spores under investigation.

2.3. Ozone treatment and analysis

Bacterial and fungal spores were exposed to different concentrations of ozone viz. 0.0, 1.0, 3.0, 5.0, 7.0 and 9.0 g/m^3 for 1 minute. Microbial survival was expressed as the log spores per ml.

2.4. Electron microscopy

The samples were coated by gold sputter coated (SPI-Module) and examined by Scanning electron microscopy (JEOL-JSM-5500 LV) by using high vacuum mode at the Regional Center of Mycology and Biotechnology, Cairo, Egypt.

3. Results

3.1. Effect of ozone on *Bacillus subtilis* ATCC 6633 spores

Treatment of spore suspension with different ozone concentrations, of 1.0, 3.0, 5.0, 7.0 and 9.0 g/m^3 for 1 minute, reduced spore counts by 5.4 x 10^7 until become undetected at ozone concentrations of 7.0 and 9.0 g/m^3, respectively. Results in table (1) illustrate the sporicidal effect of ozone, since the total count of *B. subtilis* ATCC 6633 spores decreased obviously when the concentrations of ozone increased (Fig.1).

Table 1. Treatment of *B. subtilis* (ATCC 6633) spores with different Ozone concentrations for 1 min. at 37 ºC

Ozone Conc. (g/m^3)	Viable spores (CFU/ml)	Log spores/ml
0.0	5.4 X 10^7	7.7
1.0	2.2 X 10^5	5.3
3.0	4.3 X 10^3	3.6
5.0	5.8 X 10^2	2.7
7.0	0.0	0.0
9.0	0.0	0.0

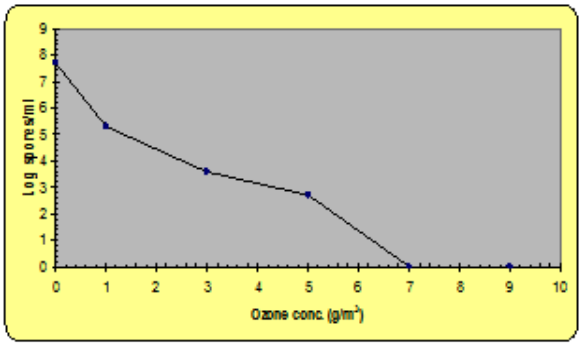

Figure 1. Inactivation of *B. subtilis* ATCC 6633 spores, when treated with different Ozone concentrations for 1 min. at 37 ºC

3.2. Mechanism of action of ozone on *Bacillus subtilis* ATCC 6633 spores

Inactivation of bacteria by ozone is a complex process because ozone attacks numerous cellular constituents including proteins, unsaturated lipids and respiratory enzymes in cell membranes, peptidoglycan in cell envelopes, enzymes and nucleic acids in the cytoplasm, proteins and peptidoglycan in spore coats. Correlation between susceptibility of *B. subtilis* ATCC 6633 spores to ozone gas may reflect the mechanism of spore inactivation. Spores, treated and untreated with ozone, were examined by scanning electron microscope (SEM). Obviously, these micrographs revealed damage to the surface layer of ozone-treated spores (Fig.2).

Figure 2. Scanning electron microscopic micrograph of *B. subtilis* ATCC 6633 spores; untreated (A) or treated (B) with ozone conc. (5.0 g/m^3) at 37 ºC for 1 min. Note that the surface layer and the outer spore coat are the structures most apparently damaged by ozone treatment

3.3. Ozone effect on *Aspergillus brasiliensis* ATCC 16404 spores

The survival rate of *Aspergillus brasiliensis* ATCC 16404 was decreased as ozone concentrations increased. Increasing ozone concentrations are more effective against spores of *Aspergillus brasiliensis* ATCC 16404 as shown in table (2). It was found that ozone concentration of 9.0 g/m^3 has a lethal effect on *A. brasiliensis* ATCC 16404 spores during 1 minute exposure (Fig.3). From the table it can be seen that moderate doses of ozone are sufficient to achieve significant microbial reductions.

Table 2. Reduction of the survival rate of *Aspergillus brasiliensis* ATCC 16404 at different ozone concentrations for 1 min at 37 ºC

Ozone conc. (g/m^3)	Viable spores (CFU/ml)	Log spores/ml
0	3.7 x 10^5	5.57
1	2.2 x 10^5	5.34
3	4.8 x 10^3	3.68
5	3.4 x 10^2	2.53
7	0.5 x 10^2	1.71
9	0.0	0.0

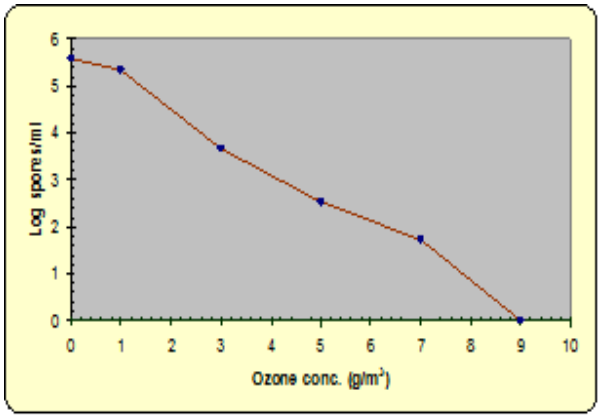

Figure 3. Effect of different ozone concentrations on the spores of *A. brasiliensis* ATCC 16404 during 1 min exposure at 37ºC

3.4. Mode of action of ozone on *A. brasiliensis* ATCC 16404 spores

Scanning Electron micrograph appearance of the spores (Fig.4) showed observable changes in the fine structure of the fungal spores. Ozone, in our study, damaged the outer layer of spore as well as the inner layer. It is suggested that the vast majority of these spores lost their viability. Ozone and its

produced free radicals play a part in this inactivation mechanism but there is no consensus on which of them is more decisive. As shown in fig.(4) the fungal spore is damaged by spore disruption or disintegration leading to leakage of the spore contents. Our present study on ozone supports the notion that ozone can kill spores by degrading outer spore components, and exposing the spore core to the action of the oxidizing agent.

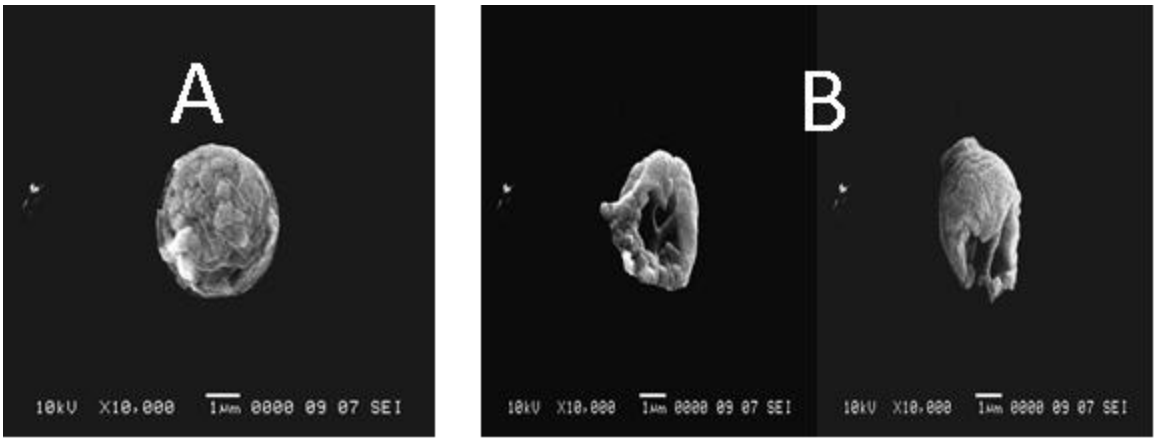

Figure 4. Scanning electron microscopic micrograph of *Aspergillus brasiliensis* ATCC 16404 spores; untreated (A) or treated (B) with ozone. Ozone-treated spores were exposed to ozone conc. (7.0 g/m^3) at 37 ºC for 1 min. Note that the fungal spore is damaged by spore disruption or disintegration leading to leakage of the spore contents

4. Discussions

The purpose of this study was to investigate the potential of using ozone gas to kill microbial (fungal and bacterial) spores. The results achieved during this study show that ozone may work as a sanitizing agent for disinfection and may be even for sterilization, although a higher ozone concentration was needed to get a major killing effect on *Aspergillus brasiliensis* ATCC 16404. An experiment to see the effect of ozone on fungal and bacterial spores in a short time (1 min.) was performed, and the killing effect was high. In just 1 min. almost all of the tested microorganisms including the very tolerant *Aspergillus brasiliensis* spores were inactivated. The experiment performed to see how well ozone at different concentrations could be used for eliminating microbial spores in water. This result is significant in terms of the fact that the used ozone gas has a sporicidal effect on the bacterial and fungal spores under investigation. This work is quite complete and agrees with that of other workers. Foegeding, (1985) found that *Bacillus cereus* spores, with removed coat, were rapidly inactivated by ozone, compared to intact spores.

Bacterial spores are known to be resistant to common antimicrobial physicochemical agents (Mathias *et al*., 2010). As compared to vegetative cells, spores are highly resistant to a wide range of toxic chemicals (Setlow, 2006). The spore's first line of defense is the coat, the multiple protein layers of which act as a chemical filter. The researcher concluded that the spore coat is a primary protective barrier against ozone. Recently, Khadre and Yousef (2001b) found that spores of *Bacillus subtilis* treated with aqueous ozone showed heavily disrupted outer spore coats.

Researches that are carried out on fungi indicated that the mode of action of ozone on fungi is not certain. Since ozone attacks cellular membranes of higher plants, perhaps fungal membranes could be similarly affected. If that is true, exposed conidial membranes could experience decreased differential permeability. Perhaps ozone increases conidiophore respiration, resulting in prematurely formed and nonviable conidia. Ozone exposure of conidia to 0.30 ppm for two 6-hr periods totally inhibited their ability to infect detached leaves (McKeen, 1974). Ozone inactivation of pathogenic fungi (*Aspergillus niger*) was studied by Coronel *et al*. (2002). Ozone at certain doses may inhibit, directly or indirectly, enzyme activity of the fungus thus resulting in less maceration of cells and possibly decreased infection. A comprehensive study has shown the effectiveness of ozone as a germicidal agent against a wide range of pathogenic organisms like bacteria, protozoa, fungal and bacterial spores (Kim and Yousef, 1999). Kottapalli *et al*. (2005) indicated significant reduction in *Fusarium* survival rates upon treatment with gaseous ozone.

Ozone is a potent oxidizing agent that can be used for disinfection in the food industry (Rice *et al.*, 2000). Low concentrations of ozone and shorter

contact times are necessary compared to other weaker oxidizers such as chlorine, mono-chloramine and chlorine dioxide (DeMers and Renner, 1992). Ozone also is a potent sanitizer with promising applications in the modern food industry. The sanitizer is effective against a wide spectrum of microorganisms, and it can be used in an environment-friendly manner. Currently, ozone is the most likely alternative to chlorine and hydrogen peroxide in food applications.

Knowledge of the disinfection mechanism of pathogens is important for optimizing kill efficiency and minimizing undesired effects on surroundings and on higher-level multicellular organisms. Disinfectants can be classified into two groups according to whether the kill mechanism originates from inside the pathogen or outside. Ozone may oxidize various components of cell envelope including polyunsaturated fatty acids, membrane-bound enzymes, glycoproteins and glycolipids leading to leakage of cell contents and eventually causing lysis (Khadre *et al*., 2001).

RNA of microorganisms is degraded into protein subunits by ozonation (Kim *et al*., 1980). Roy *et al*. (1981) indicated that the primary mode of fungal spore inactivation by ozone appears to be nucleic acid damage. Ozone degradation of nucleic acids was also studied by Shinriki *et al*. (1981). Several authors referred to enzyme inactivation as an important mechanism by which ozone kills cells. Takamoto *et al*. (1992) observed that ozone decreased enzyme activity in *E. coli* at a greater degree in case of cytoplasmic α-galactosidase than in case of the periplasmic alkaline phosphatase. More recent work has shown that ozone treatment does not destroy spores by causing DNA damage, but affects spore germination by damaging the inner membrane of the spore's coat (Young, 2000). Ozone is shown to have produced single and double-strand breaks in plasmid DNA and to open up circular plasmid DNA (Hamelin, 1985). Ozone treatment also decreased transcription activity of plasmid DNA (Mura and Chung, 1990). Ozone has also been shown to cause mutation in *E. coli*, however, ozone was considered to be a weak mutagen (Dubeau and Chung, 1982). Franco (2005) found that ozonation of RNA causes its denaturation. Also, ozone showed antifungal activity against *Aspergillus fumigatus* (Geweely, 2006).

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Diversity of Medicinal Plants in the Biospherical Reservation Areas of Iran

(A Case Study of the protected area of Miankaleh)

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Abstract: Awareness of people concerning the side effects of chemical drugs has caused an increasing interest in traditional medicine. This study was carried out to gather and identify medicinal plants, their curative effects and the part of them which is used from the reservation area of Miankaleh. The region under study has an area of 68800 hectares situated 12 kilometers north of the city of Behshahr and northwest of the city of Gorgan. During numerous visits to the area, plants were gathered and, after their identification using specialized references of medicinal plants, the part used and the curative effects of the plants were determined. Results obtained showed that out of a total of 43 families, 125 genera, and 155 species found in the region, 33 families, 52 genera, and 61 species (39% of all the species) belonged to medicinal plants, among which the class Asteraceae with 6 species and the class Chenopodiaceae with 5 species had the most medicinal species. The most used parts of the plants were the leaves with 31%, the whole plants with 19%, and the roots with 15%.

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Key words: Miankaleh, Medicinal plants, Boispherical reservation area, traditional medicine

1. Introduction

Since ancient times, plants have been one of the first and most available resources usable for treating illnesses, and throughout history there has always been a close relationship between man and plants, and the medicinal effects of plants and their uses have been known by everybody (UNESCO, 1996). Today, chemical medicines, because of their harmful and irreversible effects on people, are slowly being replaced by medicines extracted from plants (Banerjee, 2002). More than 422000 species of flowering plants have been reported from all over the world about 5000 species of which are used for medicinal purposes. There are about 8000 plant species out of which 569 genera and 2300 species are medicinal (Mozaffarian, 2005).

There is a considerable and growing interest in herbal medicines in the world since, according to international statistics, the value of trade in herbal medicines enjoys a yearly growth of 12- 15% de Silva (1997). It is worth mentioning that in Germany, which is a big center of chemical drugs production, more and more herbal medicines are used by patients and prescribed by doctors (Pande et al., 2004). Medicinal plants are so important that pharmaceutical experts search among plants to find medicines of the 21st century and these experts believe that plants are the solution to medical problems of the future. Use of traditional and medicinal plants in developing countries is widely attracting attention as the main basis for maintaining health (Nazir et al, 2010). For this same reason, identification, preservation, and sustainable management of these valuable resources are necessary (Hamilton 2003). This study was carried out with the purpose of directly accessing herbarium samples, identifying the medicinal plants of the region, finding out the parts of the plants used, and what illnesses they are used for.

2. Materials and Methods

2.1. The Characteristics of the Region Studied

The protected region of Miankaleh, which consists of two wet and dry ecosystems, has an area of 68800 hectares, 18000 hectares of which belong to the arid part and the rest to the wetland part. The region is 60 kilometers long and its width varies from 5 to 12 kilometers. Miankaleh is 12 kilometers north of Behshahr and northwest of Gorgan, with a longitude of 53 35′ 54.2″ east and a latitude of 36 45′ 64.55″ north and an altitude of 21-22 meters below sea level, at the extreme southeast of the Mazandaran Sea (the Caspian Sea). To the north of Miankaleh lies the Caspian Sea and to the south and to the east, there is the Gulf of Gorgan.

Miankaleh consists of two wet (the Gorgan Gulf and the Miankaleh wetlands) and dry ecosystems, includes a complex of beaches, marshes, pools and lowlands. It is a suitable place for various plant species due to its unique features as a habitat, and is the only remaining one of the wooded coastal and wetland types of the coast of the Caspian Sea.

2.2. Climate

Climate is the result of various elements of weather, is formed after a long time in adaptation to the geographical position of each region, and plays an important role in relation to the renewable resources of the region. By acquiring a complete awareness of the capacities and limitations the climatic factors impose on each region, we can make optimal use of these resources. The weather in this region is affected by the climate of the southern plains and is considered wet temperate, according to climate classification.

2.3. Soil

The soil in the region is alkaline and it has a light (sandy or sandy silt) and deep texture. The available phosphorous is low to medium. The land area in this region is composed of low sand dunes and seaside beaches with a little to medium expanse of rolling lands .In areas near the coast, the topsoil is salty due to the salty sea water which causes the establishment of halophytic plants**.**

2.4. The Geomorphology of the Region

The coastal provinces of Mazandaran and Gilan were formed during the Quaternary and after the glaciation periods with the substantial decrease in the water level of the Caspian Sea. The formations in this region are limited to the Quaternary and include sediments relating to the Cenozoic era. Sediments in the region are sandy, calcareous, fine-grained, contain a little clayey soil, and are completely different from the sediments in Gorgan, which contain mineral clay soils.

2.5. The Vegetative Cover of the Region

In general, life forms in various plant communities are different from each other, and in fact it is this very difference that forms the basis of the structure of plant communities. In all, 179 species and sub-species were identified in the wildlife protected area of Miankaleh, most of which belong to the classes Asteraceae, Poaceae, and Fabaceae. Many of the classes found in the region have only one genus and one species. The dominant plants in the wildlife protected area of Miankaleh belong to the Iranian-Turani core and type which makes up 26.1 percent of the plants in the region. The European, the Siberian, and the Mediterranean types comprise 7.5, 3.7 and 26.1 percent of the plants in the region, respectively.

3. Methodology

The plant samples were gathered from the region and identified at the herbarium of the Agricultural Sciences and Natural Resources University of Sari. Information such as the Persian names, the parts of the plants used, and usages of the plants was obtained by using references found at the university library. This information is shown in table 1.

4. Discussion and Conclusions

Results of the study showed that, with reference to the floristic list, there are 43 classes, 125 genera and 155 species in the region, out of which 33 classes, 52 genera and 61 species belong to medicinal plants (Zargari, 1985-1991). The classes Asteraceae with 6 species and Chenopodiaceae with 5 species included the most number of medicinal species. The parts of the plants used most were the leaves (in 27 species), the whole plant (16 species), and the roots (13 species). The other parts used in the plants mentioned were seeds, bark, flowers, flower bearing browses, tubers, rhizomes, mental, and tree buds (Prajapati, 2003).

Table 1. The Plant Parts Used in the Species Present in the Protected Area of Miankaleh

Se. No	Scientific Name	Class	Form	The Part Used
1	*Heliotropium europaeum*	Boraginaceae	Tr	Leaves, flower bearing browses, seeds
2	*Circium arvense*	Compositeae	Tr	Roots
3	*Artemisia annua*	Compositeae	Tr	Aerial parts
4	*Anthemis cotula*	Compositeae	He	The whole plant
5	*Xanthium spinosum*	Compositeae	Tr	The whole plant
6	*Xanthium strumarium*	Compositeae	Tr	The whole plant
7	*Cichorium intybus*	Compositeae	He	The whole plant especially the leaves and the roots
8	*Chenopodium botrytus*	Chenopodiaceae	Ch	flower bearing browses
9	*Chenopodium albom*	Chenopodiaceae	Tr	Leaves , seeds
10	*Chenopodium murale*	Chenopodiaceae	Tr	Leaves
11	*Salsola kali*	Chenopodiaceae	Tr	The whole plant
12	*Salicornia herbacea*	Chenopodiaceae	Tr	Sap
13	*Capsella Bursa-pastoris*	Cruciferae	Tr	The whole plant
14	*Convolvulus arevensis*	Convolvulaceae	Tr	The whole plant
15	*Cyperus rotundus*	Cyperaceae	Cr	Roots ,tubers
16	*Stellaria media*	Caryophyllaceae	Tr	The whole plant
17	*Euphorbia turcomanica*	Euphorbiaceae	Tr	Leaves
18	*Granium rotundifolium*	*Geraniaceae*	*He*	*Stem*
19	*Erodium cicutarium*	*Geraniaceae*	*Tr*	*Seeds*
20	*Cynodon dactylon*	*Gramineae*	*Cr*	*The whole plant*
21	*Phragmites australis*	*Gramineae*	*He*	*Rhizomes ,roots*
22	*Hypericum perforatum*	*Hyperiaceae*	*He*	*Flower bearing browse*
23	*Linum album*	*Linaceae*	*Ch*	*Seeds*
24	*Mentha pulegium*	*Labiateae*	*Tr*	*The whole plant*
25	*Marrubium vulgae*	*Labiateae*	*He*	*The whole plant*
26	*Lycopus europaceus*	*Labiaceae*	*He*	*Shoots*
27	*Malva silvestris*	*Malvaceae*	*Cr*	*Leaves ,Flowers*
28	*Malva neglecta*	*Malvaceae*	*Cr*	*Flowers*
29	*Morus alba*	*Moraceae*	*He*	*Leaves, Bark ,Roots*
30	*Ficus carica*	*Moraceae*	*Ph*	*Sap, Stem*
31	*Oxalis corniculata*	*Oxalidaceae*	*Tr*	*The whole plant*
32	*Anagalis arvensis*	*Primulaceae*	*Tr*	*The whole plant*
33	*Samolus valerandi*	*Primulaceae*	*Tr*	*Leaves*
34	*Rumex acetosella*	*Polygonaceae*	*Ch*	*Leaves*
35	*Rumex crispus*	*Polygonaceae*	*Ch*	*Leaves ,Roots*
36	*Polygonum hydropiper*	*Polygonaceae*	*Cr*	*The whole plant*
37	*Portulace oleraceae*	*Portulaceae*	*Tr*	*Shoots*
38	*Plantago psyllium*	*Plantaginaceae*	*Tr*	*Leaves*
39	*Plantago major*	*Plantaginaceae*	*Cr*	*Leaves , Roots , Seed*
40	*Plantago lanceolata*	*Plantaginaceae*	*Tr*	*Leaves ,Roots ,Seeds*
41	*Punica granatum*	*Punicaceae*	*Ph*	*The whole plant ,Sap*
42	*Ranunculus sceleratus*	*Ranunculaceae*	*Tr*	*Sap*
43	*Ranunculus muricatus*	*Ranunculaceae*	*Tr*	*Sap*
44	*Paliurus spina christi*	*Rhamnaceae*	*Tr*	*Roots ,Leaves*
45	*Potentilla reptance*	*Rosaceae*	*Cr*	*Rhizomes, Roots, Leaves*
46	*Mespilus germanica*	*Rosaceae*	*Ph*	*Fruit, Leaves*
47	*Crataegus sp.*	*Rosaceae*	*Ph*	*Flower , Bark*
48	*Ailanthus altissima*	*Simarubaceae*	*Ph*	*Bark, Roots*

49	*Salix alba*	*Salicaceae*	*Ph*	*Bark, Branches, Leaves mental*
50	*Datura stramonium*	*Solanaceae*	*Tr*	*Leaves, Seeds*
51	*Solanum nigrum*	*Solanaceae*	*Tr*	*Leaves, flower bearing browses*
52	*Pimpinella anisum*	*Umbelliferae*	*Ch*	*Fruit*
53	*Foeniculum vulgare*	*Umbelliferae*	*He*	*Roots, Leaves, Fruit*
54	*Urtica dioica*	*Urticaceae*	*Ch*	*Leaves, Roots, Sap*
55	*Urtica urens*	*Urticaceae*	*Ch*	*Shoots, Roots*
56	*Verbena officinalis*	*Verbenaceae*	*He*	*Shoots*
57	*Viola odorata*	*Violaceae*	*Cr*	*The whole plant*
58	*Ulmus minor*	*Ulmaceae*	*Ph*	*Secondary, bark*
59	*Celtis australis*	*Ulmaceae*	*Ph*	*Leaves, Roots, Leaf buds*
60	*Peganum harmala*	*Zygophyllaceae*	*Tr*	*Seeds*
61	*Tribulus terrestris*	*Zygophyllaceae*	*Tr*	*Roots, Fruit, Leaves*

Cr: Criptophyte, Ch: Chomophyte, Ph: Phanerophyte, Tr: Trophyt, He: Hemophytee

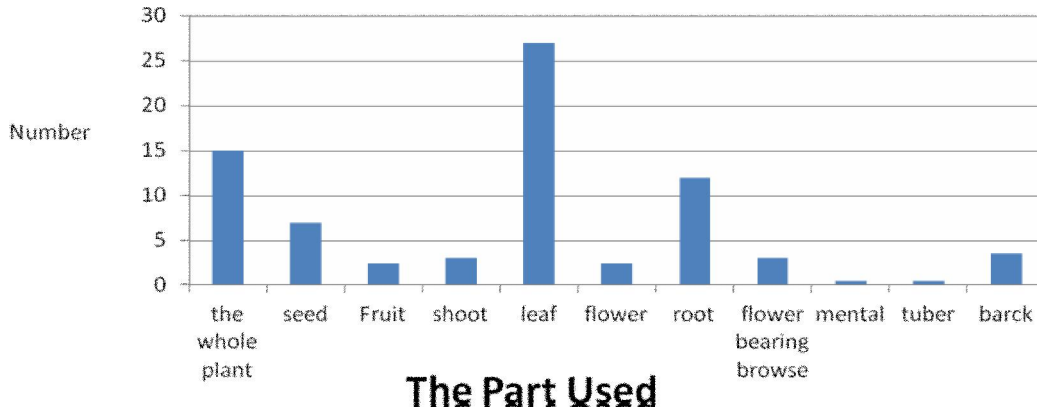

Figure 1. Parts of plants used from the species present in the protected area of Miankaleh.

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Comparative Study and Feed Evaluation of Sprouted Barley Grains on Rice Straw Versus Tamarix Mannifera on Performance of Growing Barki Lambs in Sinai

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Abstract: In arid and semi arid areas Tamarix mannifera (Tm) was considered one of the principal feed resources , rice straw (Rs) one of agriculture wastes produced in a large amount but they have low nutritive value so several treatments were applied to ameliorate the utilization of Tamarix and rice straw. The objective of this study was to investigate the effect of sprouted barley on Tm , Rs and mixture of them. Thirty five growing femal Barki lambs of about four months old with an average live body weight (L.B.W) of 16 $\pm$ 0.5kg were divided into five treatments (7 animals each) to receive one of the following experimental roughages: treatment T_1 : rice straw (Rs) ad-lib (untreated) as control; T_2:dried Tamarix ad-lib(Tm)as control ;T_3 : sprouted barley grains on rice straw ad-lib (BRs) ; T_4 : sprouted barley grains on driedTamarix ad- lib (BTm) ; T_5 : sprouted barley grains on 50 % Rs + 50 % Tm ad-lib (BRs+ BTm) . The experimental growing trial lasted for about 180 day. All animal treatments were fed 60% of total energy requirement as concentrate feed mixture (CFM). At the end of the growing trial five digestibility trial were conducted to evaluate the digestibility of the experimental roughages. Results showed that the treatments with sprouted barely increased CP, Ash and NFE while DM, OM, EE, CF, NDF, ADF and ADL contents, were decreased. Sprouted barely on Tamarix (BTm) or rice straw (BRs) revealed a significant ($P \leq 0.05$) improvement in OM, CP, EE and cellulose digestibility with an insignificant higher in CF, NDF and hemicellulose digestibility. Nutritive values expressed as TDNg/Kg B.W. and DCP% increased significantly ($P \leq 0.05$) with treatments T_2, T_3 and T_4 than untreated T_1 (Rs) and T_5 (Tm). Also, ewes fed the treated roughages retained higher ($P \leq 0.05$) nitrogen values than untreated treatments. Ewes fed sprouted barely had significantly higher ($P \leq 0.05$) values of total volatile fatty acids (VFA), ruminal ammonia (NH3- N) concentration, serum total proteins. Albumin and urea, was insignificantly increased, while serum globulin and creatinin were insignificantly decreased GOT, GPT activity than untreated roughages. The highest ($P \leq 0.05$) value of average daily gain , feed conversion (g feed/ g gain) and economical feed efficiency were recorded for T_4. However the lowest ($P \leq 0.05$) values were recorded for T_1. In conculusion we can produce green fodder by utilizing dried Tamarix and rice straw by simple methodology using crop sprouts (barley).

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Key words: Tamarix , rice straw , sprouted barley , sheep ,growth , rumen and blood parameters.

1. Introduction:

In Egypt there is a large amount of agricultural wastes produced annually, after harvesting of grains. One of these wastes is rice straw which produced in an average of 3.5 million ton on year (Khattab *et al.,* 2009). Rice straw is of poor nutritive value for ruminants related to its low protein content, high fiber content and low palatability. Abig amount of rice straw is disposed by burning , so, air pollution increased which reflect on human health. Few attempts were tried to improve nutritive value of rice straw (Ibrahim *et al*., 2001, El- Tahan *et al*., 2003 and Mohammadi *et al*., 2007).

Halophytes are considered as an important source of nutrients for most desert ruminants. some of them are less or unpalatable. Kandil and El-Shaer (1990) reported that Tamarix mannifera and other range plants containe high level of neutural detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) which could be depressed the voluntary intake and nutritive value and they appeared to be less or unpalatable halophytic species. Forage intake is related to fiber digestibility because intake is reduced when indigestible fiber is increased in the digestive tract. (Mertens., 1993).

Many investigators treated range plants by several chemical and physical treatments to improve their nutritive values (El-Essawy 2008., Fayed *et al*., 2008, and Fayed 2009).

Sprouting activities in the seeds have many changes as in seed protein converted to essential amino acids, carbohydrates are converted to sugars and fats are converted to essential fatty acids. These activities increase as a result of increasing enzymes levels (Chavan and Kadam 1989). Due to their activities enzymes, sprouts are much easier to be

digested than dry seeds. (Goodwin and Mercer 1993). The objective of the present work is to study the effect of using dried Tamarix mannifira and rice straw as media for growing barely seeds to produce green fodder in dried seasons to increase the nutritive value, palatability of Tamarix and rice straw. Green fodder was fed to growing sheep to study their effects on growth, digestibility, some rumen and blood parameters of sheep.

2. Material and Methods:

Animals and management:

This study was conducted in Ras Sudr Research station, south Sinai Governorate and lasted for six months. Thirty five female Barki lambs of four months old and average 16±51 kg live body weight were divided randomly into five equal groups. A feeding experiment followed by a metabolism trial was conducted. Animals were weighed on biweekly basis. Nutrient requirements were adjusted to the changing in the body weight every two weeks. At the end of the experimental feeding four animals from each group were randomly selected for the metabolism trial, fifteen day adaptation period followed by 5 days collection period. During the collection period, fecal and urine samples were collected daily (10% by weight of daily samples). At the end of collection period of the metabolism trial, rumen, liquor was sampled by stomach tube at 0, 3, 6, 9 hours after feeding, blood samples were taken from jugular vein at 0, 6 hours after feeding.

Experimental feed:

Dried Tamarix mannifera was collected and chopped into 2-3 cm and rice straw also, was chopped into 2-3 cm, soaked in tap water over night and used as bedding media.

Production method for seed sprouts was tray method as described by Mohammadi et al., (2007) using about 10 cm thich layer of chopped rice straw (Rs) or Tamarix mannifera (Tm) as a sprouting media. Barley grains were washed and soaked in tap water and stored in a dark area for 12 hr. (overnight) to allow for initial germination. At the end of soaking period soaked seeds were spread evenly on the top of Tamarix (Tm) or Rs media. Germination period on the media surface lasted about 10 days to get shoot sprouts, shoot length was 10- 15 cm, barley seeds were used at 20% density of roughage (rice straw and Tamarix)

Animals were fed concentrate feed mixture (CFM) to cover 60% of maintenance energy requirements according to Kearl (1982) and the roughage portion was left free choice for animals :

The tested treatments were as follows:

T_1 : CFM + rice straw (Rs)

T_2: CFM + Tamarix (Tm)

T3: CFM + sprouted barely grains on rice straw (BRs)

T_4: FM + sprouted barley grains on Tamarix (B Tm)

T_5: CFM + sprouted barley grains on 50 % (Rs) + 50 % (Tm).

Analysis:

The proximate constituents of feeds, feed refusals, feces and total nitrogen in urine were determined according to A.O.A.C.(1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin ADL were determined according to Goering and Van Soest (1970). Rumen total volatile fatty acids (TVFA,s) were analyzed according to Warner (1964), ammonia nitrogen according to A.O.A.C. (1990).

Blood samples were collected from jugular vein, serum was obtaind after centrifugation at 3000 r.p.m., stored at–20^0 c till analysis and used to determine total serum protein according to (Armstrong and Carr 1964), albumin according to (Doumas., et al 1971), globulin (was obtained by substracting the albumin values from the total proteins) creatinine according to (Henry 1965), urea according to (Patton and Crouch (1977).. Blood serum analysis was conducted using Jenway Spectrophotometer (UK) and using kits purchazed from Human Co. (Germany).

Statistical analysis:

General linear model procedure was used for statistical analysis using SAS (1998). The used design was one way analysis. Duncan's multiple tests (1955) were applied for comparison of means.

3. Results and Discussion:

Chemical composition of the tested rations:

As shown in Table (1) cleared that Dry matter (DM) content was lower in treated than untreated rice straw (Rs) and Tamarix (Tm). While rice straw had higher DM than Tamarix. Organic matter (OM) content was higher in rice straw than those for Tamarix which may due to the increase in ash content of Tamarix. The crude protein (CP) content in Rs or BRs was lower by 62.45, 26.78 than that in Tamarix, respectively. Also, BRs, BTm and 50% BRs + 50% BTm were higher in CP %, nitrogen free extract (NFE) and ash% and lower in ether extract (EE) and crude fiber (CF) contents. These results are in the same line noticed by Ibrahim *et al.*, (2001) . The CF% in BTm was lower by 42.3 than Tm and was lower in BRs by 33.2% than Rs . However, BRs + BTm had the medium level of CF than rice straw or Tamarix alone . Natural detergent fiber (NDF) , acid detergent fiber (ADF) , acid

detergent lignin (ADL) and Hemicellulose percentage were higher in untreated and treated rice straw than in treated and untreated Tamarix resp. , while NDF, ADF, ADL, cellulose and Hemicellulose percentage were lower in BRs and BTm than untreated and treated rice straw and untreated Tamarix. This finding may be attributed to increase of the activity of sprouted barley hydrolytic enzymes and lead to improvements in chemical composition of rice straw and Tamarix. Similar results were obtained by Chavan and Kadam (1989).

Table (1): Chemical composition of the experimental rations (as DM basis)

Items	CFM	Rs	Tm	BRs	BTm	50% BRs + 50%BTm
DM	93.80	91.20	85.28	85.63	81.31	86.98
OM	88.98	80.66	75.17	77.85	72.65	75.27
Ash	11.02	19.34	24.83	22.15	27.35	24.73
CP	11.02	3.09	8.23	8.07	11.02	9.67
CF	12.20	33.26	28.85	22.21	16.64	21.79
EE	3.27	2.01	2.44	1.55	2.16	1.92
NFE	62.39	42.30	35.65	46.02	42.83	41.89
NDF	36.05	71.31	64.21	59.57	51.40	56.23
ADF	24.64	53.14	50.15	40.85	38.58	40.22
ADL	5.42	14.61	12.05	11.89	10.13	10.36
Cellulose	11.41	38.53	38.10	28.96	28.45	29.86
Hemicellulose	19.22	18.17	14.06	18.72	12.82	16.01

CFM:concentrate feed mixture Rs: rice straw. Tm: Tamarix mannefera.
BRs: sprouted barley on rice straw. BTm: sprouted barley on dried Tamarix.
DM: dry matter. OM: organic matter. CP: crude protein. CF: crude fiber.
EE: ether extract. NFE: nitrogen free extract. NDF: natural detergent fiber.
ADF: acid detergent fiber ADL: acid detergent lignin.

Apparent digestibility and nutritive value:

As shown in Table (2) showed that DM and OM digestibility was not affected by the type of roughage while DM, OM, CP, EE and NFE digestibility were significantly ($P \leq 0.05$) higher with sprouted barley on Tamarix and rice straw (T_4, T_5 and T_3) than untreated Rs and Tm . Also, CF and ADL digestibility was insignificant higher in both BTm, BRs and mixture of them (T_5) than those of untreated, rice straw and Tamarix . These findings may be due to an increase in the enzymes of germination of barley grains which lead to increase in the nutrients digestibility. Agreement results were reported by Shipard (2005) who found that feeding sprouted grains provided animals with living feed which has a rich supply of enzymes which results in all nutritional components being highly digestible and extremely nutritious.

On the other hand, the digestibility coefficients of all nutrients were higher in untreated Tamarix (T_2) than that of untreated rice straw (T_1). This may be due to the chemical composition of Tamarix which contain more CP and EE% and lower content of CF, NDF, ADF, ADL and Hemicellulos than those of rice straw. These findings agree with that reported by Talha *et al*., (2005) who reported that the variation in the digestibility due to the change in the chemical composition and were inversely related to the content of nutrient in the diet.

The CF digestibility of T_4 was insignificantly higher than that of T_3, T_5, T_2 and T_1 in descending order.

On the other hand, the cellulose digestibility significantly ($P \leq 0.05$) increased in T_3 (BRs) followed by T_5 and T_4 respectively. NDF, ADF and Hemicellulose digestibility were insignificantly higher in T_3 (BRs) and T_4 (BTm) than the other treatments. This may be attributed to increase in the bioactive catalysts which assist in the digestion and metabolism of feeds and the release of energy. Similar findings were noticed by Shipard (2005). In general, most of nutrients digestibility was increased with sprouted barely grains on Tamarix or rice straw. Similar trends were observed by Ibrahim *et al.*, (2001) who found that the digestibility coefficients of all nutrients for rice straw + sprouted barley were higher than that of untreated rice straw.

Mean effects of dietary treatments on nutritive values of the experimental rations (Table 2) showed that Tamarix significantly ($P \leq 0.05$) increased total digestible nutrients (TDN g/kg B.W), digestible crude protein (DCP %) value by 82.5, 61.5, 70.9, 78% than that of untreated and treated rice straw, respectively. These results may be attributed to low digestibility of most nutrients of rice straw than

that of Tamarix. TDN g/kg BW or TDN % and DCP g/kg B.W. or DCP% for sprouted barley on Tamarix or rice straw were higher than those for untreated roughages. Similar results were obtained by Ibrahim *et al.* (2001).

Table (2): Digestion coefficients and nutritive values of lambs fed the experimental roughages

Items	T_1	T_2	T_3	T_4	T_5	±SE
DM %	53.83^{b}	55.16^{b}	64.01^{a}	65.83^{a}	64.67^{a}	3.14
OM %	55.92^{c}	59.65^{bc}	67.85^{b}	74.57^{a}	65.40^{b}	2.90
CP %	48.53^{c}	60.85^{b}	60.24^{b}	75.04^{a}	70.38^{a}	2.76
CF %	42.16	48.73	52.89	55.93	52.69	6.44
EE %	62.45^{c}	65.21^{c}	81.97^{ab}	83.18^{a}	76.02^{b}	3.65
NFE %	60.39^{b}	64.95^{b}	73.20^{a}	76.19^{a}	70.30^{a}	2.03
NDF %	40.37	45.86	49.41	48.66	41.47	5.41
ADF %	38.82	43.18	44.47	42.81	36.43	5.33
ADL %	12.65	14.77	17.84	18.01	18.09	3.22
Cellulose %	34.26^{c}	38.12^{c}	57.70^{a}	41.28^{b}	43.79^{b}	4.55
Hemicellulose	46.98	45.16	61.87	48.69	52.87	5.60
Nutritive- values						
TDNg/kg B.W	11.76^{c}	14.25^{b}	15.06^{b}	21.23^{a}	15.69^{b}	1.49
TDN%	47.96	49.67	60.85	63.87	59.25	2.65
DCP g/kg. Bw	1.23	1.52	2.09	2.12	1.89	0.12
DCP%	4.29^{c}	6.98_{b}	6.64^{b}	8.51^{a}	7.46^{ab}	0.38

T_1 : untreated rice straw (Rs) T_2 : untreated Tamarix (Tm) T_3 : sprouted barely on rice straw (BRs)
T_4 : sprouted barley on Tamarix (BTm) T_5 : 50 % BRs + 50 % BTm.
a,b,c Means with different superscripts in the same raw are significantly different at ($P \leq 0.05$)

Nitrogen balance**:**

As shown in Table (3); nitrogen intake (NI mg/kgB.W) was significantly ($P \leq 0.05$) higher with T_3 (3312.8) followed by T_5 (3200.0) while the lowest was recorded for T_1. The higher nitrogen intake was due to the higher dry matter intake while the lambs fed T_1, T_3 excreted more ($P \leq 0.05$) nitrogen in feces and lambs fed T_3 , T_5 had significant ($P \leq 0.05$) higher amounts of urinary nitrogen compared to T_4 and T_2 perhaps it could be attributed to the low Cp digestibility of rice straw than of Tamarix. Lambs fed BTm retained higher ($P \leq 0.05$) nitrogen than the other treatments. Nitrogen retention (NR)was higher for both BTm, BRs + BTm and BRs than untreated Tm or Rs while lambs fed Tm (T_2) retained nitrogen more than those fed Rs (T_1) which could be low in its content of nitrogen. Nitrogen retention as a percent of total nitrogen intake (NR% of NI) for T_4 was significantly ($P \leq 0.05$) higher than the other lambs fed the experimental roughages. This finding may be related to higher improvement in CP intake and its digestibility in Tamarix than rice straw (Table 2). Agreement results were reported by Fayed *et al.* (2009).

Table (3): Nitrogen utilization mg/kgB.W of femal lambs fed the experimental roughages.

Items	T_1	T_2	T_3	T_4	T_5	±SE
Nitrogen intake (NI)	2669.9^{c}	2765.2^{b}	3312.8^{a}	3004.8^{b}	3200.0^{a}	0.97
Fecal nitrogen (FN)	1599.3^{b}	951.2^{b}	1308.6^{a}	862.4^{b}	1107.2^{ba}	0.50
Urinary nitrogen (UN)	691.5^{c}	1211.2^{b}	1325.5^{a}	1183.9^{b}	1379.2^{a}	0.55
Total nitrogen excretion (TNE)	2290.8^{c}	2162.4^{b}	2634.1^{a}	2046.3^{b}	2486.4^{a}	0.63
Nitrogen retention (NR)	379.1^{c}	602.8^{b}	678.7^{a}	958.5^{a}	713.6^{a}	0.58
FN% of NI	59.91^{a}	34.38^{ba}	39.54^{ab}	28.67^{b}	34.58^{ba}	3.17
UN % of NI	25.91	43.83	40.01^{a}	39.44	43.12	3.94
NR % of NI	14.18^{c}	21.79^{b}	20.45^{b}	31.90^{a}	22.30^{b}	2.77

T_1 : untreated rice straw (Rs) T_2 : untreated Tamarix (Tm) T_3 : sprouted barely on rice straw (BRs)
T_4 : sprouted barley on Tamarix (BTm) T_5 : 50 % BRs + 50 % BTm.
a,b,c Means with different superscripts in the same raw are significantly different at ($P \leq 0.05$)

Rumen and blood parameters:

Data of rumen total volatil fatty acids (TVFA,s) (Fig 1) revealed that sprouted barely grains on Tamarix or rice straw and mixture of them T_4, T_5 and T_3 respectively, increased ($P \leq 0.05$) TVFA,s concentration in the rumen than untreated T_1 and T_2. While T_4 (BTm) had an increase ($P \leq 0.05$) in TVFA,s concentration (8.69 meq/ 100 ml) compare to T_5 and T_3 which have comparable values of TVFA,s (7.49 , 7.06 meq/ 100 ml) respectively . The lowest ($P \leq 0.05$) values of TVFA,s were showed in T_1 and T_2 (untreated) 4.02 and 4.49 meq/ 100 ml, these results are in harmony with those reported by Ibrahim *et al.*, (2001) who reported that TVFA,s concentration were higher ($P \leq 0.05$) for sprouted barely on rice straw and bagasse than untreated. The increase in TVFA,s concentration with sprouted barley may due to sprouts provide a good supply of vitamins, enzymes which serve as bioactive catalysts to assist in metabolism of feed and the release of energy (Shipard 2005). Concentration of VFA,s increased after feeding and reach its peak after 3 hr post feeding. Similar results were obtained by Fayed (2009) when he treated Tamarix and Acacia with two strains of pleurotus. While the untreated rice straw treatment (T_1) reach its peak at 6 hr post feeding. Similar trends were observed with nitrogen ammonia (NH_3 –N) concentration (Fig 2). Thus the greatest value of NH_3 –N was recorded for lambs fed T_4. This is may be due to such treatment contained high level of protein and its degradability. Where T_1 showed the lowest values of NH_3 –N.

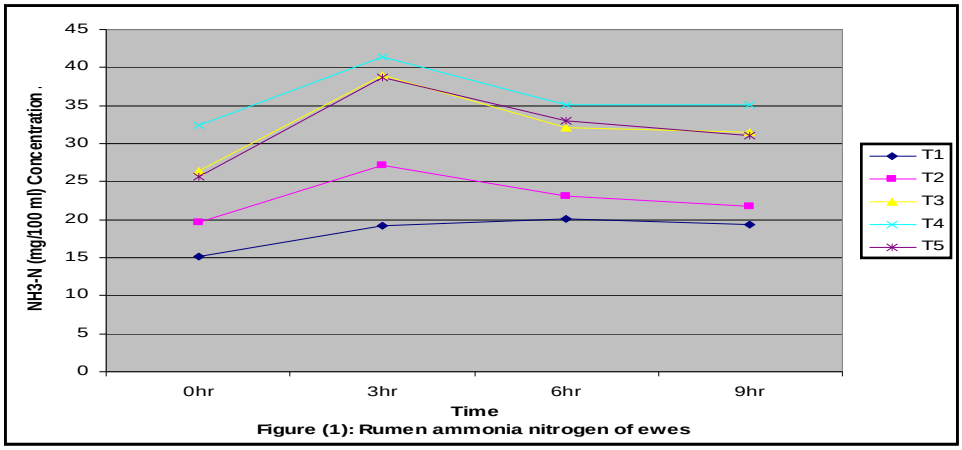

Figure (1): Rumen ammonia nitrogen of ewes

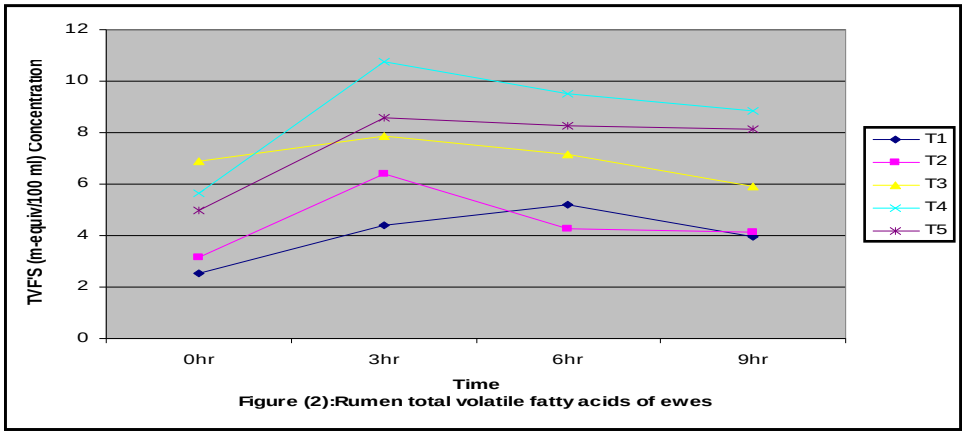

Figure (2):Rumen total volatile fatty acids of ewes

Data of Table (4) showed that total proteins concentration, Albumin- and A/G ratio were significantly elevated ($P \leq 0.05$) and globulin insignificantly affected by treatments. T_4 (BTm) and T_5 (BTm + BRs) increased ($P \leq 0.05$) serum total proteins, albumin and insignificant globulin more than the other treatments. The high level of glubulin of sprouted barely treatments may indicate good developed immunity status (Ibrahim *et al.*, 2001). A/G ration significantly ($P \leq 0.05$) increased with T_3 (BRs), while there were no significant differences between the other treatments. This was probably due to the high level of CP content in T4 and T5. This is in accordance with those reported by Kumar *et al.*, (1980) who found a positive correlation between dietary protein and plasma protein concentration.

Also, overall means of serum urea increased significantly ($P \leq 0.05$). However, serum creatinin was insignificantly increased with T4 and then T5. The lowest value of serum urea was with T1 and the lowest of creatinin was recorded for T2

Table (4): Some serum parameters of lambs fed the experimental roughages.

Items	T_1	T_2	T_3	T_4	T_5	±SE
Total protein g/d	6.31^{c}	6.79^{ac}	7.25^{b}	8.19^{a}	8.16^{a}	0.50
Albumin g/d	3.18^{c}	3.54^{b}	3.88^{b}	4.17^{a}	4.17^{a}	0.13
Globulin g/d	3.13	3.25	3.37	4.02	3.99	0.52
A/G ratio	1.02	1.09^{a}	1.19^{a}	1.04^{a}	1.08^{a}	0.14
Urea mg/d	35.73^{c}	40.56^{c}	50.70^{b}	67.30^{a}	57.68^{ab}	4.99
Creatinine mg/d	1.01	0.95	1.51	1.60	1.56	0.09

T_1 : untreated rice straw (Rs) T_2 : untreated Tamarix (Tm) T_3 : sprouted barely on rice straw (BRs)
T_4 : sprouted barley on Tamarix (BTm) T_5 : 50 % BRs + 50 % BTm.
a,b,c Means with different superscripts in the same raw are significantly different at ($P \leq 0.05$)

Feed intake:

Data of Table (5) showed that animals fed T3 (BRs) consumed total DM and roughages intake more than other treatments (1051.67 , 357.55 g/head/ day respectively) followed by T1 and T5 which have comparable values of total DM and roughages. However the lowest was T2 (Tm) followed by T4 (BTm) . Sprouted barley grains increased roughage intake by 31.79%, on rice straw and by 34.3% on Tamarix which may be attributed to increase the palatability of BRs or BTm. Similar results were reported by Eshtayeh (2004).

All treatments were fed approximately similar amounts of CFM. The results of animal performance (Table 5) showed that lambs fed T1, T3, T5, T4 and T2 gained 58.49, 90.35, 80.91, 105.72 and 68.68% of initial body weight, respectively. The greatest value of daily gain was achieved with femal lambs fed T4 (Tamarix + SB). This may be due to its nitrogen retention was the highest (369.15mg/kg.B.W). On the other hand, the significant ($p \leq 0.05$) lowest daily gain was recorded for lambs fed the untreated roughages T1 and T2 in descending order .However differences of daily gain between the treated groups (T3, T5, T4) were not significant .The increase in weight gain of lambs received barley sprouts may attributed to enhancing of microbial activity in the rumen (Tudor *et al*., 2003). Also, this observation may be due to lowest nitrogen retention and lowest digestibility of OM, CF, EE, NFE and ADF of untreated rice straw and Tamarix .

Feed conversion expressed as g feed/ g gain indicated that the lambs fed T4 (BTm) were more feed conversion as DMI (8.76), TDN (4.83) followed by T3 (BRs) and T5 (50% BRs+ 50% BTm) (12.11, 12.56, 6.61, 6.00) while the worest were the control treatments (T1, T2). These results agree with data showed by EShtayeh (2004) when sprouted barley grains on olive cake. On the other hand feed conversion was more with ewes fed BTm than ewes fed BRs. Also, lambs fed Tm were more efficient than that of Rs.

Table (5): Intake, body weights, gain and feed conversion of lambs fed the experimental roughages.

Items	T_1	T_2	T_3	T_4	T_5	±SE
No. of animals	7	7	7	7	7	-
Initial body weight (kg)	16.38	16.25	16.68	16.43	16.82	
Final body weight (kg)	25.96	27.41	31.75	33.80	30.43	
Total body gain (kg)	9.58^{c}	11.16^{bc}	15.07^{ab}	17.37^{a}	13.61^{b}	1.281
% of initial weights	58.49^{c}	68.68^{c}	90.325^{ab}	105.72^{a}	80.91^{b}	5.30
Average daily gain (gm)	53.22^{c}	62.00^{c}	83.73^{ab}	96.50^{a}	75.61^{a}	4.60
DM intake g/head/day						
Concentrate	681.45	677.23	694.12	640.15	669.88	
Roughage	248.23	134.76	357.55	205.11	245.91	
Total DMI g/head/day	929.68	811.99	1051.67	845.26	915.19	
TDN intake g/head/day	415.95	405.50	502.38	466.01	499.76	
Feed conversion						
gm feed/ gm gain						
DMI	17.37	13.10	12.56	8.76	12.11	
TDN	7.82	6.54	6.00	4.83	6.61	

T_1 : untreated rice straw (Rs) T_2 : untreated Tamarix (Tm) T_3 : sprouted barely on rice straw (BRs)
T_4 : sprouted barley on Tamarix (BTm) T_5 : 50 % BRs + 50 % BTm.
a,b,c Means with different superscripts in the same raw are significantly different at ($P \leq 0.05$)

Economical evaluation**:**

Economical efficiency was affected by type of roughages (Table 6). lambs fed sprouted barley grains on Tamarix (T_4) had better values of economical efficiency (1.71) than other experimental roughages T5, T3, T2 and T1 in descending order the values were 1.30, 1.26, 1.19 and 1.00, respectively. These results indicate that sprouted barley grains on Tamarix had minimum price for production one kilogram gain by about by 41.5, 26.3, 23.9 and 30.4% than T1, T3, T5 and T2 respectively. This may be attributed to the highest values of feed conversion as DMI, TDN/ kg gain , to that the price of rice straw was expensive than the price of collection Tamarix (Allam *et al* ., 2006) .

Table (6): Economical evaluation of lambs fed the experimental roughages.

Items	T_1	T_2	T_3	T_4	T_5	±SE
Price of feed intake h/day L.E*						
Concentrate	1.05	1.04	1.07	0.99	1.03	0.05
Roughages	0.10	0.006	0.26	0.14	0.13	0.21
Total price of feed intake	1.15	1.046	1.33	1.13	1.16	0.14
Feed cost/ daily gain L.E.						
Feed cosl/ kg gain	19.94^a	16.87^b	15.88^b	11.71^c	15.34^b	0.95
Economical feed fficiency**	1.00	1.19	1.26	1.71	1.30	0.03

a,b,c Means with different superscripts in the same raw are significantly different at ($P \leq 0.05$)
* Based on market price. The price of ton on DM basis was as follows: CFM, 1540, barely 1580 and rice straw, 400 L.E.
The price of 1 kg live body weight at selling time was 20 L.E.
** Economic feed efficiency expressed as the ratio between the price of total live body weight gain and the price of feed consumed to that gain.

4. Conclusion:

It could be concluded that in arid season we can produce green fodder by utilizing dried salt plants and rice straw by simple methodology using crop sprouts. Rice straw by-product could employ to produce forage feed instead of being burned and causing pollution.

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Study of the Right Liver Lobe Size /Albumin Ratio as a Noninvasive Predictor of Oesophageal Varices Compared to: Spleen Size, Platelet Count and Platelet Count/Spleen Diameter Ratio in Post Hepatitis C Virus Liver Cirrhosis in Egypt

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Abstract: Back ground and aim: Hepatitis C Virus (HCV) is considered the most common aetiology of chronic liver disease in Egypt.Portal hypertension is a major complication of liver cirrhosis, and leads to the development of portosystmic shunts. Oesophageal varices are the most important among these shunts. Bleeding from oesophageal varices is the most serious complication of cirrhosis, with a high risk of death. The prevention of variceal bleeding is very important, non-selective beta blockers and prophylactic band ligation decrease the risk of bleeding by 50%. The current guide lines recommend screening of all cirrhotic patients by endoscopy, to identify patients at risk of bleeding so prophylactic treatment should be started to them. But repeated endoscopic examinations are unpleasant for patients, and carries high cost impact and more burden on endoscopic units, while only 50% of cirrhotic patients have esophageal varices, and up to 30% have large varices. For these reasons many non-invasive predictors for the presence and size of varices have been studied. The aim of this study is to evaluate prospectively the right liver lobe size /albumin ratio and to compare it with spleen size, platelet count and platelet count/spleen diameter ratio as noninvasive predictors of oesophageal varices in post hepatitis C virus liver Cirrhosis in Egypt. Patients and methods: This prospective study included one hundred patients with post hepatitis C virus liver Cirrhosis. All studied subjects underwent a detailed history taking, clinical examination and a biochemical workup, including total bilirubin, aspartate aminotransferase, alanine aminotransferase, serum albumin, prothrombin activity, complete blood count and viral markers for hepatitis C and hepatitis B viruses. Child-Pugh score was calculated for all patients. An upper gastrointestinal endoscopy and abdominal ultrasound were performed for all patients. The platelet count to spleen diameter ratio and the right liver lobe to albumin ratio were calculated. Results: All the 4 predictors showed high statistically significant correlation with the presence and the grade of oesophageal varices (P values <0.001) Among the 4 noninvasive predictors the platelet count/spleen diameter ratio gave the highest accuracy at a cut-off value of 1326.58 (sensitivity 96.34% and specificity 83.33%) followed by the right liver lobe/albumin concentration ratio at a cut-off value of 44.2 (sensitivity 91.46% and specificity 77.78%) followed by the spleen size at a cut-off value of 131.5mm(sensitivity 90.24% and specificity 83.33%) then lastly the platelet count at a cut-of value of 131000/mm^3(sensitivity 84.15% and specificity 83.33%).

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Key words: Noninvasive predictors of oesophageal varices, the right liver lobe/albumin ratio, the platelet count/spleen diameter ratio, Oesophageal varices, Post HCV liver cirrhosis.

1. Introduction:

Egypt has a very high prevalence of hepatitis C virus (HCV) and a high morbidity and mortality from chronic liver disease [(1)]. HCV is considered the most common aetiology of chronic liver disease in Egypt, where prevalence of antibodies to HCV (anti-HCV) is 10-fold greater than in the United States and Europe [(2)].

Portal hypertension is a major complication of liver cirrhosis, and leads to the development of portosystmic shunts. Oesophageal varices are the most important among these shunts due to its clinical effects and play a major role in transforming the disease from a pre-clinical to a clinical phase. Longitudinal studies have shown that oesophageal and/or gastric varices eventually develop in all cirrhotic patients [(3, 4)] and that once they have developed they tend to increase in size and to bleed [(4)]. The yearly rate of development of new varices is about 5–10% [(3, 5)]; the rate of growth of varices from small to large ranges between 5% and 30% in different studies [(5–8)]. Bleeding from oesophageal varices is the most serious complication of cirrhosis, with a high risk of death [9].The mortality from each

episode of variceal bleeding is17%-57% [4, 10, 11]. On endoscopic examination the presence of red spots on the varices equals high risk of bleeding which is also related to the size of varices [12, 13].

The prevention of variceal bleeding is very important, non-selective beta blockers and prophylactic band ligation decrease the risk of bleeding by 50% ([14, 15]. It is recommended that all cirrhotic patients should undergo endoscopic screening for the presence of varices [16–21], patients who has large or medium sized varices should be treated to prevent bleeding.

Patients who don't have varices and with compensated cirrhosis should repeat endoscopy every 2-3 years, and every 1-2 years for those with small varices [17]. It is also recommended for patients with decompensated cirrhosis to repeat endoscopy every 1 year even if there are no varices [17, 19]. But repeated endoscopic examinations are unpleasant for patients, and carries high cost impact and more burden on endoscopic units, while only 50% of cirrhotic patients have esophageal varices, and up to 30% have large varices. For these reasons many non-invasive predictors for the presence and size of varices have been studied.

This study attempts to evaluate prospectively the right liver lobe size /albumin ratio and to compare it with spleen size, platelet count and platelet count/spleen diameter ratio as noninvasive predictors of oesophageal varices in post hepatitis C virus liver Cirrhosis in Egypt

2. Materials and methods:

This prospective study included one hundred patients with post hepatitis C virus liver Cirrhosis who were under investigations and treatment at the Gastroenterology & Hepatology outpatient clinics or those who were admitted to the Internal Medicine departments of the Cairo university hospitals between January 2009 and March 2010.

Diagnosis of cirrhosis was based on physical findings, laboratory investigations and imaging findings. Patients who previously underwent injection sclerotherapy, band ligation, surgery for oesophageal varices, and those who were receiving beta blockers were excluded from the study. All patients with liver cirrhosis due to causes other than HCV were also excluded.

All studied subjects underwent a detailed history taking, clinical examination and a biochemical workup, including total bilirubin, aspartate aminotransferase, alanine aminotransferase, serum albumin, prothrombin activity, complete blood count and viral markers for hepatitis C and hepatitis B viruses. Child-Pugh score was calculated for all patients using the 5 parameters (ascites, albumin, bilirubin, prothrombin activity and encephalopathy) [22]. An upper gastrointestinal endoscopy and abdominal ultrasound were performed in all patients.

The right liver lobe diameter in the midclavicular line and the maximum spleen bipolar diameter were measured and the values were recorded. The platelet count to spleen diameter ratio and the right liver lobe to albumin ratio were calculated.

All endoscopies were performed in a single endoscopy unit by an experienced endoscopist and a grading classification I – IV was used [23]. Grade I was used for varices in the level of mucosa, grade II for varices smaller than 5 mm filling less than 1/3 of the oesophageal lumen, grade III for varices larger than 5 mm filling more than 1/3 of the oesophageal lumen and grade IV for varices occupied more than 2/3 of esophageal lumen.

All the data were recorded, analyzed and correlated.

Data were statistically described in terms of range, mean ± standard deviation (± SD), median, frequencies (number of cases) and percentages when appropriate. Comparison of quantitative variables between the study groups was done using Mann Whitney *U* test for independent samples when comparing 2 groups and Kruskal Wallis analysis of variance (ANOVA) test with Mann Whitney *U* test for independent samples as posthoc multiple 2-group comparisons when comparing more than 2 groups. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. Accuracy was represented using the terms sensitivity, specificity, +ve predictive value, -ve predictive value, overall accuracy, the likelihood ratio of a positive test and the likelihood ratio of a negative test. Receiver operator characteristic (ROC) analysis was used to determine the optimum cut off value for the studied diagnostic markers. A probability value (*p* value) less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs Microsoft Excel 2007 (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

3. Results:

48 men and 52 women were included in the study, all with post HCV liver cirrhosis. The main clinical characteristics of all patients are shown in table 1.

The mean values of platelet (PLT) count, spleen diameter, PLT count/spleen diameter ratio and the right liver lobe diameter/albumin concentration ratio were correlated to the presence and grade of

varices and they all highly significantly correlated to the presence and grade of varices as shown in table 2 and table 3.

The sensitivity, specificity and accuracy of PLT count, spleen diameter, PLT count/spleen diameter ratio and the right liver lobe diameter/albumin concentration ratio as noninvasive predictors of oesophageal varices were studied by applying the ROC curve to detect the cut off values with the best sensitivity and specificity.

Among the 4 noninvasive predictors the platelet count/spleen diameter ratio gave the highest accuracy at a cut-off value of 1326.58 followed by the right liver lobe/albumin concentration ratio at a cut-off value of 44.2 followed by the spleen size at a cut-off value of 131.5mm then lastly the platelet count at a cut-of value of 131000/mm^3 as shown in table 4 and figures 1-5.

Table 1: showing the patients clinical characteristics

Main clinical characteristics of all patients	
Total number	100
Gender (M/F)	48/52
Age (mean ±SD)	49.23 ± 7.996
Age (range)	20 - 70
Child class.(A/B/C)	20/31/49
Varices present (yes/no)	82/18
Grade of varices(I/II/III/IV)	7/15/35/25
Varices type (small/large)	22/60
Mean PLT count,mm^3 (mean ±SD)	117070 ± 66145.883
Mean spleen diameter, mm (mean ±SD)	150.92 ± 23.371
Mean PLT count/spleen ratio(mean ±SD)	843.262 ± 616.250
Mean right liver lobe diameter, mm (mean ±SD)	147.74 ± 4.263
Mean albumin concentration , gm./dl (mean ±SD)	2.543 ± 0.9699
Mean right lobe/ albumin ratio (mean ±SD)	66.578 ± 23.735

Table 2: Correlation of all parameters in patients with and without varices

Varices presence		PLT count	Spleen diameter	PLT/spleen ratio	Right lobe/albumin ratio
No	Mean	215,055.56	121.22	1,838.389	41.187
	SD	69,772.295	13.584	707.1507	8.8507
Yes	Mean	95,560.98	157.44	624.820	72.152
	SD	41,519.919	19.745	301.3943	22.3031
P		<0.001	<0.001	<0.001	<0.001

Table 3: Correlation between all predictors and grades of varices

Grade of varices		PLT count	Spleen diameter	PLT/spleen ratio	Right lobe/albumin ratio
I	Mean	167,428.57	136.29	1,204.285	42.192
	SD	59,969.040	15.966	364.5529	5.6384
II	Mean	99,466.67	149.87	668.750	56.054
	SD	37,015.183	16.296	246.9659	16.0342
III	Mean	96,000.00	160.60	600.033	72.150
	SD	31,167.479	14.136	199.6266	15.5372
IV	Mean	72,480.00	163.48	470.914	90.201
	SD	25,932.798	24.395	237.9270	20.3913
P		<0.001	0.007	<0.001	<0.001

Table 4: Comparison of accuracy of the 4 parameters in predicting the presence of oesophageal varices

Predictor	AUROC	Cut off point	Sensitivity (%)	Specificity (%)	(+)ve PV(%)	(-)ve PV(%)	Accuracy (%)	LR+	LR-
PLT count	0.912	131000	84.15	83.33	95.83	53.57	84.00	5.05	0.19
Spleen size	0.934	131.5	90.24	83.33	96.10	65.22	89.00	5.41	0.12
PLT count/spleen ratio	0.927	1326.58	96.34	83.33	96.34	83.33	94.00	5.78	0.04
Right liver lobe/Albumin conc. ratio	0.912	44.22	91.46	77.78	94.94	66.67	89.00	4.12	0.11

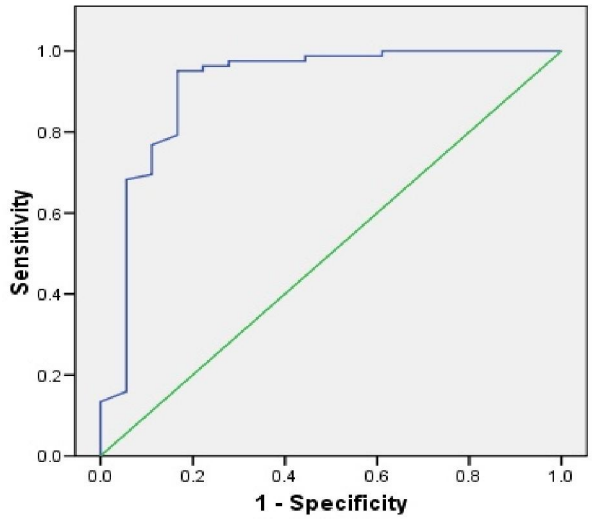

Figure 1: ROC curve for sensitivity and specificity of platelet count for the prediction of varices

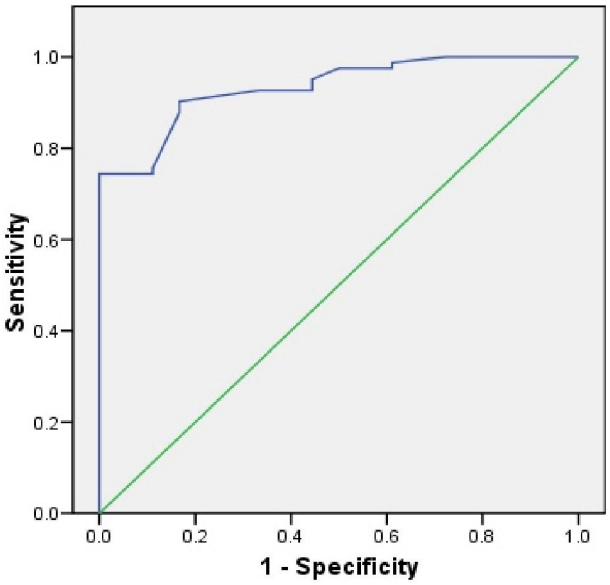

Figure 2: ROC curve for sensitivity and specificity of spleen size for the prediction of varices.

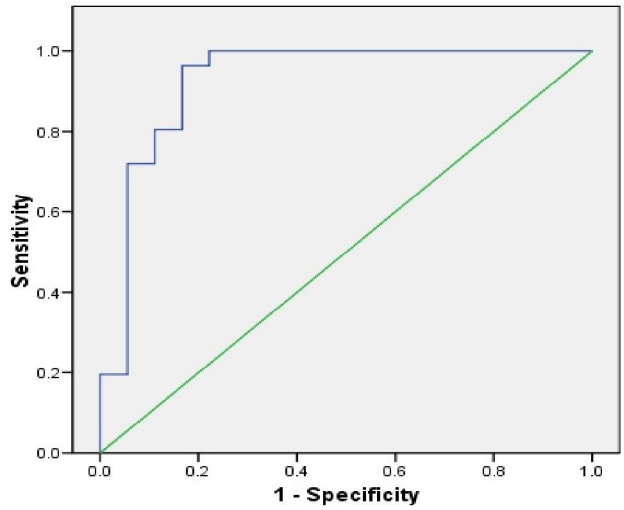

Figure 3: ROC curve for sensitivity and specificity of platelet count/spleen diameter ratio for the prediction of varices.

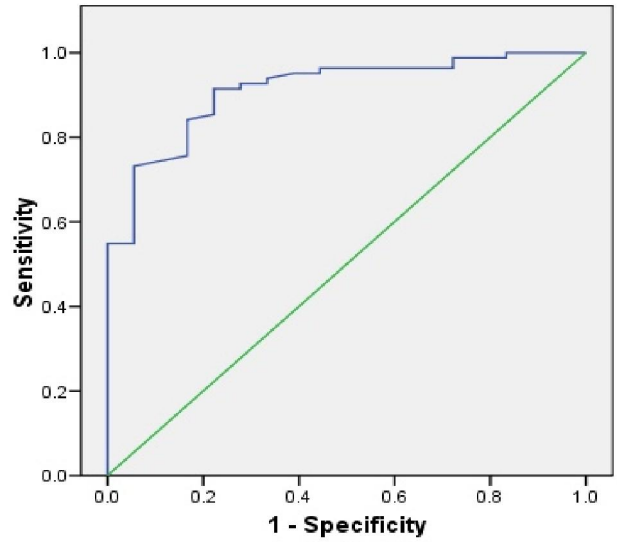

Figure 4: ROC curve for sensitivity and specificity of right liver lobe size/albumin concentration ratio for the prediction of varices.

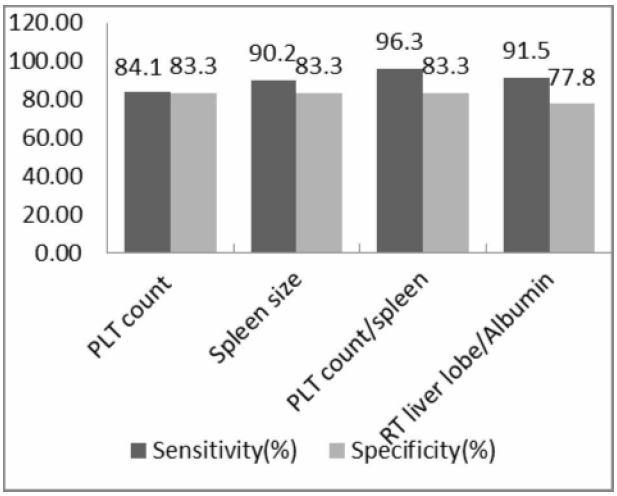

Figure 5: Comparison between sensitivity & specificity of the 4 parameters in predicting the presence of oesophageal varices

4. Discussion:

Bleeding oesophageal varices is still the leading cause of death in patients with cirrhosis. In recent studies, mortality rates vary between 11% and 20% within six weeks of the bleeding episode [24-27].

Endoscopy is still the gold standard method for diagnosis of oesophageal varices and is recommended every two to three years in cirrhotic patients without varices, and every one to two years in patients with small varices [14, 28, 29].Several studies have been performed to find noninvasive parameters that can predict the presence of oesophageal varices in liver cirrhosis to reduce the cost and burden on endoscopy units [28].

The prevention of bleeding from oesophageal varices is an important goal. Identification of patients who are at risk of variceal bleeding is the first step in prevention of bleeding so the patients can be selected to start prophylactic treatment.

The prevalence of oesophageal varices among cirrhotics is variable, ranging from 24% to 80% [30]. The value of diagnosing oesophageal varices by a noninvasive predictor is to save endoscopy to patients who have high probability of having varices.

In the present study as shown in tables 2-4 and figures 1 and 2 like many other previous studies [31-37] have shown that platelet count and spleen diameter correlate well with the presence of oesophageal varices. However, in cirrhotic patients, the presence of thrombocytopenia may be due to several factors other than portal hypertension, as shortened mean platelet lifetime, decreased thrombopoietin production or myelotoxiceffects of hepatitis C virus [38]. The presence of splenomegaly in cirrhotic patients is mainly related to portal hypertension.

In 2003Giannini et al [28] introduced the use of the platelet count/spleen diameter ratio as a predictor of oesophageal varices. This ratio links thrombocytopenia to splenomegaly to introduce a variable that takes into consideration that thrombocytopenia is mainly due to hyperslenism secondary to portal hypertension. In his study with a cut-off value of 909 the sensitivity was 100% and specificity was 93%. In 2006 Giannini et al [39] reported the results of a multicenter study to validate the use of platelet count/spleen diameter ratio in the prediction of oesophageal varices. In this study the cut-off value of 909 showed sensitivity 92% and specificity 67%.Many studies [23, 39- 42] have been done using different best cut-off values to investigate this parameter as a noninvasive predictor for oesophageal varices.

In the present study the cut-off value of 1326.58 for the platelet count /spleen diameter ratio was used which showed sensitivity 96.34% and specificity 83.33% as shown in table 4 and figure 3.
In 2007 Alempijevic et al [24] investigated the right liver lobe diameter/albumin concentration ratio as a noninvasive predictor of oesophageal varices and at a cut-off value of 44.25 the sensitivity was 83.1% and the specificity was 73.9%. In the present study at a cut-off value of 44.22 for the right liver lobe diameter/albumin concentration ratio, the sensitivity was 91.46% and the specificity was 77.78% as shown in table 4 and figure 4.

5. Conclusion:

Among the noninvasive parameters studied in this study, the platelet count/spleen diameter ratio had the highest accuracy for diagnosing oesophageal varices (sensitivity 96.34% and specificity 83.33%). For the right liver lobe diameter/albumin concentration ratio, the sensitivity was 91.46% and the specificity was 77.78% and can be considered as a noninvasive predictor of oesophageal varices that can provide accurate information as well as the platelet count/spleen diameter ratio.

The use of the 4 studied predictors in this study can help the physicians to restrict endoscopy on those who are highly suspected to have oesophageal varices to start the prophylactic therapy and not to use the endoscopy for all the patients.

Of course endoscopy still is the gold standard for the diagnosis of oesophageal varices, but the use of the noninvasive predictors specially platelet count/spleen diameter ratio and the right lobe liver size/albumin concentration ratio will be of a

great help to reduce the number of endoscopies in patients with post hepatitis C virus liver cirrhosis in Egypt. More studies are required in a larger sample of post hepatitis C cirrhosis patients for validation of the right lobe liver size/albumin concentration ratio as a noninvasive predictor of oesophageal varices as well as the platelet count/spleen diameter ratio and to determine a cut-off value that can be safely recommended for the noninvasive diagnosis oesophageal varices.

The limitation of the present study includes: relatively small number of patients, liver biopsy was not done and the diagnosis of cirrhosis was based on clinical and laboratory results.

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12/27/2010

Strategies of Rural Development in Shoushtar Township of Iran (Applying SWOT method)

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Abstract: The purpose of this research was using SWOT for identifying strategies of rural development in Shoushtar township of Iran. SWOT technique used for clarifies strengths, weaknesses, opportunities, and threats of rural area in Shouahtar Township, Iran. The population of study was people of rural area of Shoushtar. The sample size (n=110) determined by Cochran formula and selected by random sampling. Based on the results, external (opportunities and threats) and internal (strengths and weaknesses) factors that affected on situation of rural area were evaluated. Based on the participant's idea, each item ranked and importance ratio coefficient identified. Based on the results the score of external and internal factor were 2.05 and 1.71. Also, SWOT results indicated important strategies for rural development were: SO_1: Using new technology for increasing productivity, SO_2: Planting new crops with high economic value, ST_1: Designing developmental plan for development markets, ST_2: Environmental and natural sustainability, ST_3:Development of agricultural policy regarding efficiency use of possibilities, WO_1: Using new technology for public services, WO_2: Development of extension program for HRD, WT_1: Development practices for contracting equality in social and economical condition and WT_2: Development of agricultural policies for productivity in poor farmers practices.

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Keywords: SWOT, External Factor Evaluation, Internal Factor Evaluation

1. Introduction

A new method for strategic programming is applying SWOT (Strengths, Weaknesses, Opportunities and Threats) matrix. Rural practices analysis is a critical part of the strategic management planning process. The strengths, weaknesses, opportunities, and threats (SWOT) framework is proposed by many as an analytical tool which should be used to categorize significant factors both internal and external to the rural development practices (Ommani, 2010., Pickton and Wright, 1998., Zoller and Bruynis, 2007).

A SWOT analysis can help researchers gain insights into the past and think of possible solutions to existing or potential problems. For a SWOT analysis to work well, every member of team (family and/or employees, lawyer, accountant, and insurance agent) should be involved in the process (USDA, 2008).

The SWOT analysis is used to provide a clear assessment of the situation. It identifies the fields and activities that have higher potential for further development and improvement for Shoushtar Township. This technique is considered as a simple, yet effective, mean to assess the current situation by analyzing four key points:

S: What are the strengths and advantages of rural area of Shoushtar?

W: What are the weaknesses and disadvantages?

O: What are the opportunities that of rural area of Shoushtar can exploit?

T: What are the threats and obstacles that can negatively affect the development of rural area of Shoushtar ?

Riston (2008) pointed out benefits of external and internal analysis include:

-Increasing managerial awareness of environmental changes.
-Improving resources allocation decisions.
- Facilitating risk management.
- Acting as an early warning system.
- Focusing attention on the primary influences on strategic change.

2. Material and Methods

SWOT technique used for clarifies strengths, weaknesses, opportunities, and threats of rural area in Shouahtar Township, Iran. The population of study was people of rural area of Shoushtar. The sample size (n=110) determined by Cochran formula and selected by random sampling.

In the study, following phases were used:

I) Designing external and internal factors matrix.
II) Analyzing SWOT matrix.
III) Designing Quantitative Strategic Programming Matrix (QSPM).
IV) Priorities identified strategies.

3. Results

Analysis external and internal factors: At this phase of research, external (opportunities and threats) and internal (strengths and weaknesses) factors that affected on situation of rural area were evaluated. Based on the participant's idea, each item ranked and importance ratio coefficient identified. Based on the results the score of external and internal factor were 2.05 and 1.71.

External Factor Evaluation (EFE): EFE matrix method is a strategic-management tool often used for assessment of current business conditions. The EFE matrix is a good tool to visualize and prioritize the opportunities and threats that a business is facing.

The EFE matrix process uses the five steps :

List factors: The first step is to gather a list of external factors.

Divide factors into two groups: opportunities and threats. Assign weights: Assign a weight to each factor. The value of each weight should be between 0 and 1 (or alternatively between 10 and 100 if you use the 10 to 100 scale). Zero means the factor is not important. One or hundred means that the factor is the most influential and critical one. The total value of all weights together should equal 1 or 100.

Rate factors: Assign a rating to each factor. Rating should be between 1 and 4. Rating indicates how effective the firm's current strategies respond to the factor. Rating captures whether the factor represents a major threat (rating = 1), a minor threat (rating = 2), a minor opportunity (rating = 3), or a major opportunity (rating = 4). If you use the rating scale 1 to 4, then strengths must receive a 4 or 3 rating and weaknesses must receive a 1 or 2 rating.

Multiply weights by ratings: Multiply each factor weight with its rating. This will calculate the weighted score for each factor.

Total all weighted scores: Add all weighted scores for each factor. This will calculate the total weighted score for the company.

Internal Factor Evaluation (IFE) matrix: IFE matrix is a strategic management tool for evaluating strengths and weaknesses in functional areas of a business. The IFE Matrix together with the EFE matrix is a strategy-formulation tool that can be utilized to evaluate how a company is performing in regards to identified internal strengths and weaknesses of a company. The IFE matrix can be created using the following five steps:

Key internal factors: The first step is identify strengths and weaknesses.

Weights: IFE matrix, assign a weight that ranges from 0.00 to 1.00 to each factor. The weight assigned to a given factor indicates the relative importance of the factor. Zero means not important. One indicates very important.

Rating: Practitioners usually use rating on the scale from 1 to 4. Rating captures whether the factor represents a major weakness (rating = 1), a minor weakness (rating = 2), a minor strength (rating = 3), or a major strength (rating = 4).

SWOT is the first step of planning and helps planners to focus on key subjects. SWOT method is a key tool for businesses to formulate strategic plans.

SWOT matrix including four strategies groups: How are used strengths to take advantage of opportunities?, How are reduced the weaknesses by taking advantage of opportunities?, How are used strengths to reduce the impact of threats? and How are addressed the weaknesses that will make these threats a reality?

Table 1: Internal Factors Evaluation Matrix (EFEM) and External Factors Evaluation Matrix (IFEM) regarding rural area of Shoushtar Township

Internal Factors	**Weight**	**Rating**	**Weighted Score**
Strengths			
Favorable geographical position	0.12	4	0.48
An environment rich in natural resources	0.09	4	0.36
The capacities and experience in developing the agriculture industry	0.10	3	0.30
Availability of own machinery	0.09	3	0.27
Weaknesses			
A weak infrastructure	0.16	1	0.16
The lack of expertise in managing	0.15	1	0.15
Low level of public services	0.14	1	0.14
Low level of human resources management activities	0.15	2	0.30
Total Weighted Score	1		1.71
External Factors	**Weight**	**Rating**	**Weighted Score**
Opportunities			

Implementing new technologies	0.10	3	0.30
The development of the tourism industry	0.11	4	0.44
Planting new crops	0.12	3	0.36
The development of agricultural industry	0.12	3	0.36
Threats			
Lack of, or uncertain, market	0.14	1	0.14
The migration of local intellectuals	0.13	1	0.13
Poor agricultural policies and the high level of bureaucracy	0.13	1	0.13
Inequality in social and economical condition	0.15	1	0.15
Total Weighted Score	1		2.01

1: How are used strengths to take advantage of opportunities?

SO Strategies

	Strengths (S)
	Favorable geographical position
	An environment rich in natural resources
	The capacities and experience in developing the agriculture industry
	Availability of own machinery
Opportunities (O)	SO_1: Using new technology for increasing productivity SO_2: Planting new crops with high economic value
Implementing new technologies	
The development of the tourism industry	
Planting new crops	
The development of agricultural industry	

2: How are reduced the weaknesses by taking advantage of opportunities?

WO Strategies

	Weaknesses (W)
	A weak infrastructure
	The lack of expertise in managing
	Low level of public services
	Low level of human resources management activities
Opportunities (O)	WO_1: Using new technology for public services WO_2: Development of extension program for HRD
Implementing new technologies	
The development of the tourism industry	
Planting new crops	
The development of agricultural industry	

3: How are used strengths to reduce the impact of threats?

ST Strategies

	Strengths (S)
	Favorable geographical position
	An environment rich in natural resources
	The capacities and experience in developing the agriculture industry
	Availability of own machinery
Threats (T)	ST_1: Designing developmental plan for development markets. ST_2: Environmental and natural sustainability ST_3:Development of agricultural policy regarding efficiency use of possibilities.
Lack of, or uncertain, market	
The migration of local intellectuals	
Poor agricultural policies and the high level of bureaucracy	
Inequality in social and economical condition	

4: How are addressed the weaknesses that will make these threats a reality?

WT Strategies

	Weaknesses (W)
	A weak infrastructure
	The lack of expertise in managing
	Low level of public services
	Low level of human resources management activities
Threats (T)	WT_1: Development practices for contracting equality in social and economical condition.
Lack of, or uncertain, market	
The migration of local	

intellectuals	WT_2: Development of agricultural policies for productivity in poor farmers practices.
Poor agricultural policies and the high level of bureaucracy	
Inequality in social and economical condition	

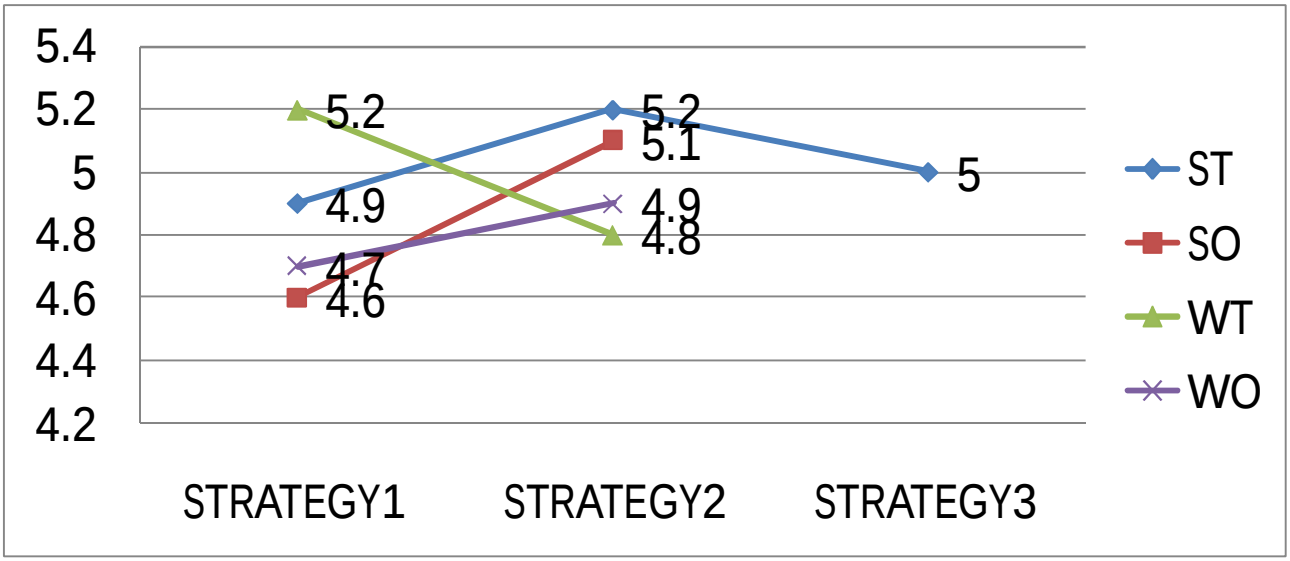

Figure 1: Prioritize of strategies based on Quantitative Strategic Planning Matrix score (QSPM)

SWOT results indicated important strategies for rural development were: SO_1: Using new technology for increasing productivity, SO_2: Planting new crops with high economic value, ST_1: Designing developmental plan for development markets, ST_2: Environmental and natural sustainability, ST_3:Development of agricultural policy regarding efficiency use of possibilities, WO_1: Using new technology for public services, WO_2: Development of extension program for HRD, WT_1: Development practices for contracting equality in social and economical condition and WT_2: Development of agricultural policies for productivity in poor farmers practices.

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Serum Levels of cytokines in poly-transfused patients with Beta-Thalassemia major: Relationship to splenectomy

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Abstract : Beta thalassemia is the most common chronic haemolytic anemia in Egypt. A major cause of morbidity and mortality in β-thalassemic patients is infections, assumed to be the result of immunological changes. Cytokines production by immune cells is superior representative of phenotypes and functions of lymphocytes, but results of previous researches are not satisfactory and in some cases are controversial, due to differences in their experimental designs. So the aim of this study was to determine the possible defect, we investigated the cytokine IL-2 and IL-8 productions by blood cells of β-thalassemic patients. The study was conducted on fifty one patients with homozygous beta-thalassemia major (23 of them were splenectomized group 1), who attending the Haematology Clinic, New Paediatric Hospital, Faculty of Medicine, Cairo University. Beside 17 healthy subjects served as control, with the same age matched group. All subjects were subjected to: full clinical examination, complete blood counting, liver function tests, and renal function tests. Determination of IL-2 was done by an immunoenzymometric assay for the quantitative measurement (Biosource IL-2 EASIA kit), and Determination of IL-8 by AviBion Human Interleukin-8 ELISA kits. The result showed that, there were significant increase **(P** <0 .05) in the serum level of IL-8 among group 1(mean level was 526.4 ± 65.7 U/ml) as compared to control group (mean level was 208.67 ± 35.53 pg/ml) as well as group 2 (mean level was 438.21 ± 58.063 pg/ml). Also group 2 had significant increase **(P** <0 .05) in the serum level of IL-8 as compared to control group. While, the levels of serum IL-2 showed no significant changes **(P** >0 .05) between the thalassaemic groups as well as the control group. In conclusion, the study revealed that beta-thalassemia major patients had increased level of IL-8 which was more prominent in splenectomized patients. The potential role of IL-8 and the interactions between different cytokines in thalassaemic patients require further investigation. Multi-transfusions could be responsible for a change in circulating cytokines that could contribute to a state of partial immune deficiency in beta-thalassaemic patients, which is more prominence among the splenectomized patient.
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Key words: thalassemia major- IL-2, IL-8- splenectomy

1.Introduction:

Thalassemia is an inherited autosomal recessiveblood disease. In thalassemia, the genetic defect results in reduced rate of synthesis of one of the globin chains that make up hemoglobin. Reduced synthesis of one of the globin chains can cause the formation of abnormal hemoglobin molecules. Beta-thalassemias are a group of hereditary blood disorders characterized by anomalies in the synthesis of the beta chains of hemoglobin resulting in variable phenotypes ranging from severe anemia to clinically asymptomatic individuals. The total annual incidence of symptomatic individuals is estimated at 1 in 100,000 throughout the world (Galanello & Origa, 2010). Beta-thalassemia is characterized by reduced synthesis of the hemoglobin subunit beta (hemoglobin beta chain) that results in microcytic hypochromic anemia, an abnormal peripheral blood smear with nucleated red blood cells, and reduced amounts of haemoglobin A (HbA) on haemoglobin analysis. Individuals with thalassemia major have severe anemia and hepatosplenomegaly; they usually come to medical attention within the first two years of life. Without treatment, affected children have severe failure to thrive and shortened life expectancy (Geetha *et al.*, 2004).

The haemoglobin disorders are the most common clinically serious single gene disorders in the world. Beta thalassemia is the most common chronic haemolytic anemia in Egypt. So, it was estimated that 1,000/1.5 million per year live births will suffer from thalassemia disease in Egypt (total live births 1,936,205 in 2006) El-Beshlawy & Youssry 2009. A major cause of morbidity and mortality in β-thalassemic patients is infections, assumed to be the result of immunological changes (Ghollam *et al.*, 2006).

Patients with thalassemia major commonly develop hypersplenism after 5-10 years of regular blood transfusions. As a result of splenic enlargement transfusion requirement steadily increase, accelerating the influx of toxic iron. Splenectomy is one form of the management of thalassemia disease Nasim et al., 2000. Splenomegaly tends to develop later and splenectomy can be postponed until the second decade of life or later. The commonly adopted criteria for splenectomy is a blood consumption greater than 50% above the mean requirement of the splenectomized population, i.e. more than 200-250 ml/kg/year of pure red cells, to maintain a pre-transfusion Hb around 9 g/d L (LISA *et al.*, 2008)

Infectious complications constitute the second most common cause of mortality and a main cause of morbidity among major β-thalassemia patients. These could be the results of functional alteration in the immune system due to multiple blood transfusions. Recent studies on immune competence in β-thalassemia have revealed numerous quantitative and functional defects involving T and B lymphocytes, immunoglobulin production, neutrophils and macrophages, as well as the complement system (Farmakis *et al.*, 2003) suggested to be the results of iron overload and. There are also reports showing the phenotype of lymphocytes changes and increase in the circulating level of C-reactive protein occurs among the post-splenectomized patients, while there is no significant changes among non-splenectomized.

Cytokines production by immune cells is superior representative of phenotypes and functions of lymphocytes, but results of previous researches are not satisfactory and in some cases are controversial, due to differences in their experimental designs. For example; Lombardi and colleagues in 1994 showed that serum level of IL-2 and IL-6 are undetectable or within the normal range in all their β-thalassemic patients, while results obtained by Aggeli and co-workers in 2005 indicate an increase in the circulating level of IL-6 among these patients. It is known that interleukin-6 (IL-6) and interleukin-8 (IL-8) are important components of the pro-inflammatory response. The plasma levels of these cytokines may be relevant in the pathophysiology of beta-thalassemia (Oztürk *et al.*, 2001). For this purpose, the aim of this study plasma level of IL-2 and IL-8 cytokine were measured among both non-splenectomized and splenectomized thalassaemic patients.

1. Patients and Methods:

The study was conducted on fifty one patients with homozygous beta-thalassemia major (they were diagnosed by clinical examination and hemoglobin electrophoresis), who were attending the Haematology Clinic, New Paediatric Hospital, Cairo University, for receiving blood transfusion and treatment. Beside 17 age and sex matched healthy person served as control group. The patients were divided into two groups:

1- Group 1(+ ve splenectomy)

Comprised (23) patients 12 Male (52.2 %) and 11 female (47.8 %), they were blood transfusion dependent and had splenectomy since 10 month. Their ages ranged from 4 to 19 years.

2- Group 2 (-ve splenectomy)

Comprised (28) patients 18 Male (64.3 %) and 10 female (35.7%), they were blood transfusion dependent and without splenectomy. Their ages ranged from 3 to 18 years.

1.1. Blood sampling

Ethically, patients were informed and written statements of their agreements were collected before sample collection. Peripheral fasting venous blood samples were collected from each patient and healthy person. The blood was left to clot at room temperature to separate sera after centrifuging for 10 minutes at 3000 r. p. m. Sera were divided into several aliquots and stored at – 70°C until assay.

1.2. All patients were subjected

- Full clinical history and clinical examination
- Complete blood count: included haemoglobin concentration, hematocrite, and rectics, using Coulter counter and examination of Lishman or Wright-stained peripheral blood smears.
- Determination of serum alanine transaminase (ALT) and serum aspartate transaminase (AST) levels by using the method recommended by Committee on enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (1976). The test was performed using already commercially available kit from Boehringer-Mannhiem Company, Germany.
- Determination of serum bilirubin level by colorimetric method using available kit from Bio-Merieux Company, France (Perry *et a.l*, 1983).
- Determination of serum urea level was done by colorimetric method according to Tietz (1995) the Kit from Croma Test Company Spain.
- Determination of serum creatinine by enzymatic method for creatinine utilizes a multi-step approach ending with a photometric end-point reaction. The enzyme creatinine amidohydrolase is used to convert creatinine to creatine. Creatine is broken down to sarcosine and urea by creatine amidinohydrolase. Further enzyme linked steps with sarcosine oxidase and peroxidase yield a colored chromogen read at 545nm acoording to (Young *et al.*, 1990)

- Determination of IL-2 by an immunoenzymometric assay for the quantitative measurement (BIOSOURCE IL-2 EASIA kit). It is a solid phase Enzyme Amplified Sensitivity Immunoassay. Cat. No. KAC1241-KAC1242, BioSource Europe S.A., Rue de L'Industrie, 8 B-1400 Nivelles Belgium (ROBB, 1984).
- Determination of IL-8 by AviBion Human Interleukin-8 ELISA kits. It is an enzyme-linked immunosorbent assay for quantitative detection of human IL-8. The assay recognizes both natural and recombinant Hu IL-8. Ref. IL08001, Ani Biotech Oy, Orgenium Laboratories Business Unit, Tiilitie 3, Fin-07120 Vantaa FINLAND (Baggiolini *et al*., 1989).

1.3. Statistical analysis

The data were coded and entered in a personal computer using the Statistic packages SPSS (11.5 version) computer program. Results are expressed as mean ± standard Error. Different groups were compared using student t test and the *p* values of less than 0.05 were considered statistically significant.

2. Results

The study was conducted on fifty one patients with beta-thalassemia major, their age ranged from 3-19 years; they were divided into two groups one with splenectomy and the other without splenectomy. Besides seventeen normal healthy control subjects matched for the age and sex were enrolled in this study.

The clinical data of the patients showed, pallor in 56.5% in group 1 while 57.1 % in group 2, and typical mongoloid facies (large head, prominence of the check bone tend to obscure the base of the nose and exposed the upper teeth, frontal bossing, and protuberance of the abdomen) appeared almost in all patients as evident by clinical examination and X rays. Hepatomegaly was present in 39.1% of group 1 while it present in 39.3% of group 2 as evident by the patients abdominal ultra sonography. Splenomegaly was present in 14.3% among group 2 as shown in Table (1).

Table 2 showed the laboratory finding of the patient groups in relations to control group, the mean level of red blood count in group 1 was $3.76 \pm 0.67 \times 10^6$ cells/ml while in group 2 it was $3.04 \pm 0.8 \times 10^6$ cells/ml which were significant reduced **(**$P < 0.05$) as compared to the control group.

The mean level of haemoglobin concentrations in both groups were reduced, in group 1 it was 1 6.1 ± 0.36 g/dl while in group 2 it was 6.53 ± 0.182 g/dl which they were significant ($P < 0.05$) as compared to the control group.

As regards the total leucocytic count there were no statistical significant **(**$P > 0.05$) between the studied groups apart a slight increase in group 1, which was not significant.

Liver function tests (ALT and AST) and renal function tests (urea and creatinine) showed no significant variation ($P > 0.05$) between the studied groups, while the serum level of bilirubin were increased in the two groups of patients, the mean level of bilirubin was 1.92 ± 0.43 in group 1 and it was 1.53 ±0.11 in group 2. They were a significant increase **(**$P < 0.05$) of serum bilirubin in both groups as compared to controls, as shown in Table (2).

Table 3 showed that, there were significant increase **(**$P < 0.05$) in the serum level of IL-8 among group 1(mean level was 526.4 ± 65.7 U/ml) as compared to control group (mean level was 208.67 ± 35.53 pg/ml) as well as group 2(mean level was 438.21 ± 58.063 pg/ml). Also group 2 had significant increase **(**$P < 0.05$) in the serum level of IL-8 as compared to control group as shown in table (3) & figure (1).

Serum IL-2 levels showed no significant changes ($P > 0.05$) between the thalassaemic groups as well as the control group as shown in table (3) & figure (2).

Table (1): Demographic data and clinical finding present in the studied groups.

	Control	Group 1 (+ve)	Group 2 (-ve)
Number	17	23	28
Age(years) Range:	4-18	4-19	3-18
mean ± SE	8.27 ± 1.06	9.1.84 ± 0.81	8.7 ± 0.83
Male/Female	10/7	12/ 11	18/10
Pallor	-	13 (56.5%)	16 (57.1%)
Jaundice	-	9 (39.1%)	15 (53.7%)
Mongoloid facies	-	21 (91.3%)	27 (96.4%)
Hepatomegaly	-	9 (39.1%)	11 (39.3%)
Splenomegaly	-	-	4 (14.3 %)

- **Group1(+ve) : Patient with splenectomy.**
- **Group1(-ve) : Patient without splenectomy.**

Table (2): Laboratory finding of the patient and control groups. (Data expressed as Range & mean ± SE) and Statistical Variation.

	Control	Group 1 (+ve)	Group 2 (-ve)
Number	17	23	28
Red blood cells (x 10^6 cells/mL)	4.3 – 5.1 4.67 ± 0.33	2.5 - 4.2 3.76 ± 0.76	2.1 – 3.8 3.04 ± 0.8
Haemoglobin (g/dL)	9.9–14.1 13.0 ± 1.1	3.9 -9.5 6.1 ±0.36*****	5.1 – 8.7 6.53 ± 0.182*****
Leukocytic count (×10^9/L)	3.9 – 10.5 8.75 ± 0.12	4.9 – 11.9 9.8 ± 0.67	5.1 – 10.7 9.6 ± 0.23
Liver function tests: **ALT (U/L)**	12-42 28.27 ± 1.01	14-47 31.72 ±1.45	13-46 30.16 ± 1.72
AST (U/L)	17- 41 29.1 ± 1.71	19 – 48 31.92 ± 1.67	23 – 54 34.93 ± 1.4
Bilirubin (mg/dL)	0.2-1.01 0.7 ± 0.02	0.8-3.1 1.92 ± 0.43*	0.4 -2.8 1.533 ± 0.11*

ALT= Alanine transaminase, AST= Aspartate transanimase
*** Statistical significant compared to control group P<0.05**

Table (3): Range and Mean ± SE for Serum Interleukin 8 and Interleukin 2 Levels in the Different Studied Groups and Statistical Variation.

	Control No. 17	Group 1 No. 23	Group 2 No. 28
IL-8 (pg/ml) Range Mean ± SE	 47.76 -528.78 208.678 ± 35.53	 116.35 - 1445 526.40 ± 65.72	 77.05 – 1099 438.213 ± 58.063
P value : Group 1vs. Cont. Group 2vs. Cont. Group 1 vs. group 2		0.000219* - -	0.018364* 0.049783 -
IL-2 (U/ml) Range Mean ± SE	 0.35 - 2.33 1.686 ± 0.179	 0.29 - 3.14 1.87 ± 0.19	 0.45 -2.79 1.905 ± 0.167
P value : Group 1 vs. Cont. Group 2 vs. Cont. Group 1 vs. group 2		 0.191298	 0.095193 0.282238

***P value :- P<0.05 considered significant.**

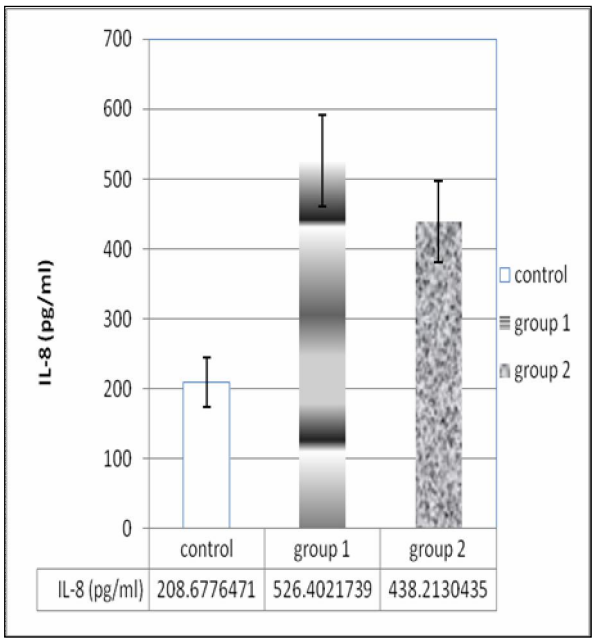

Figure 1: Mean ± S.E. for Serum Levels of Interleukin-8 (IL-8) (pg/ml.) in the Different Studied Groups

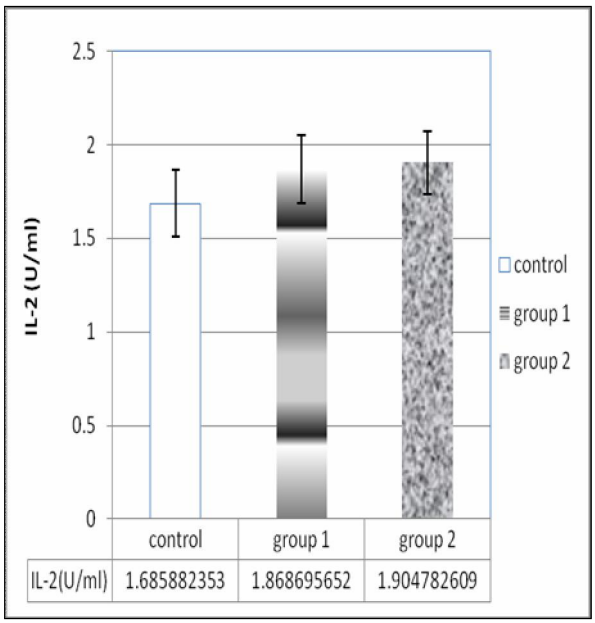

Figure 2: Mean ± S.E. for Serum Levels of Interleukins-2 (IL-2) (U/ml) in the Different Studied Groups

3. Discussion

Several, sometime contradictory immunological defects have been reported in patients with beta-thalassemia. They include: impaired activity of monocytes and neutrophils, defective activity of the complement alternative pathway, increased serum immunoglobulin levels, numerical or functional alternation of different peripheral lymphocytes and blood anomalies of serum level of cytokines. The mechanism of these abnormalities is not clarified. Factors such as splenectomy, iron overload, repeated exposure to foreign antigens at the time of blood transfusions and the use of chelating agent deferoxamine, known to have profound effects on the immune system. The role of immunologic alternations on the clinical course of beta thalassemia is not established, although they have been considered relevant to infectious episodes that these patients suffer (Consolini *et al.*, 2001).

The results of this study showed that there was significant increase in the serum IL-8 level in the thalassaemic patients with a profound increased in splenectomized patients (group 1). These increment in the serum level of IL-8 could be due to several immunological defects can be found in patients with beta-thalassaemia, among which the impairment of neurophil and macrophage phagocytic and killing functions and the production of some cytokines are the most important. It is known that interleukin-6 (IL-6) and interleukin-8 (IL-8) are important components of the pro-inflammatory response. The plasma levels of these cytokines may be relevant in the pathophysiology of beta-thalassaemia (Oztürk *et al*., 2001).

These results were in accordance with Oztürk et al study who found that plasma IL-8 levels in the patients who had blood transfusions over 100 times were significantly higher than those of under 100 times ($p < 0.05$), whereas there was no statistical difference for IL-6. Markedly increased plasma IL-6 and IL-8 levels were documented in patients with beta-thalassemia. Increased production of IL-6 and IL-8 might have contributed to abnormalities in iron metabolism and it is probably due to overstimulation of macrophages (Oztürk *et al*., 2001).

Also, Meliconi found that high IL-8 serum concentrations in the majority of beta-thalassaemic patients. A likely cause of this IL-8 production could

be ascribed to the transfusion-related continuous antigenic stimulation and iron overload with consequent macrophage activation. They concluded that macrophages (and fibroblasts) can be responsible for IL-8 production either directly or indirectly via TNFα synthesis (Meliconi *et al.*, 1993).

The cloned interleukin-8 (IL-8), also known as neutrophil-activating peptide- 1 (NAP-I), acts primarily and almost exclusively on neutrophils, stimulating chemotaxis and degranulation. This peptide can be produced by a variety of cell types, including large granular lymphocytes, macrophages, endothelial cells, fibroblasts, and synovial cek4 Activated monocytes, endothelial cells, and fibroblasts may produce IL-8 in response to exogenous and endogenous stimuli, such as lipopolysaccharide (LPS), tumor necrosis factor (TNF), and IL-I (Strieter *et al.*, 1989).

Increased serum levels of IL-8 and TNF-α were reported in homozygous polytransfused beta-thalassemia major (Uguccioni *et al.*, 1993). In this study the authors suggested that the main causes for the rise in these cytokines were macrophage activation due to iron overload and the antigenic stimulation related to chronic transfusion therapy. Since it has also been reported that during erythrophagocytosis activated monocytes may produce different cytokines to enhance their phagocytic function (Simms *et al.*, 1991) and that IL-8 may increase in response to endogenous stimuli such as tumor necrosis factor and IL-1 (Strieter *et al.*, 1989). Also, in a study done by Fausto et al., in 1995, they thought that the elevated serum IL-8 levels found in untransfused thalassemia syndromes could be related to the phagocytosis of red blood cells by hyperactive monocytes.

The result of this study showed that, there was slight non-significant increase in the serum level of IL-2 in the both groups of thalassemia as compared to control group. The reason for the low or normal serum levels of IL-2, a potent inducer of B-lymphocyte differentiation and of their capacity to synthesize immunoglobulin, despite strong stimulation of the B-immune system such as that described in our patients with b-thalassemia is not clear (Lombardi et al., 1994).

On the other hand Gharagozloo *et al.* (2008) studied the immunologic abnormalities of Iranian beta-thalassemia major patients. Their results showed that Patients with thalassemia showed significantly increased absolute lymphocyte counts compared with the control group. An increased number of activated T cells and higher levels of serum neopterin were also observed in thalassemia patients, which suggest chronic stimulation of immune system. On the contrary, T-cell proliferation and interleukin 2 (IL-2), interferon gamma (IFN-gamma), and IL-4 production were suppressed in patients compared to controls.

Also, Moshtaghi-Kashanian *et al.*,(2006), showed that IL-2 production of thalassaemic patients' groups were significantly ($p<0.01$) lower than corresponding value obtained for the control group. They concluded that, multi-transfusions could be responsible for a change in the subset of circulating lymphocytes that could contribute to a state of partial immune deficiency in beta-thalassaemic patients, which is more prominence among the splenectomized patient.

4. Conclusion

The study revealed that beta-thalassemia major patients had increased level of IL-8 which was more prominent in splenectomized patients. The potential role of IL-8 and the interactions between different cytokines in thalassaemic patients require further investigation. Multi-transfusions could be responsible for a change in circulating cytokines that could contribute to a state of partial immune deficiency in beta-thalassaemic patients, which is more prominence among the splenectomized patient. So evaluation of the serum levels of selected cytokines may be a useful tool in improving our knowledge about these functional immunological defects in b-thalassemia.

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Strategies for Retaining Youth in Rural Communities

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Abstract: The identify factors affecting on migration youths to urban centers is very important to rural program development. For develop strategies that attract and keep youth in rural communities, reasons youth migrate to urban centers must be closely examined and identified. The research method employed was correlative-descriptive. The population consisted rural youth in Shoushtar township of Khuzestan province in Iran. A random sample of rural youths (n=360) was selected. Data collected were analyzed using the Statistical Package for the Social Sciences (SPSS). Appropriate statistical procedures for description (frequencies, percent, means, and standard deviations) were used. The main result of the study revealed that top reasons by youth for moving to urban centre including: employment, education, family-related and to get away. Also the top eight strategies for retaining youth to rural communities were: Improve career opportunities**,** Provide work experience opportunities **,** Improve opportunities for education after high school**,** Improve opportunities for social activities**,** Improve access to amenities**,** Promote the advantages of rural living**,** establishment of youth advisory committees establishment of youth priorities for local government**,** Promote youth involvement in community decision making. From a development perspective, the youth are the future for any country and the world. The potential of youth to transform rural communities needs to be recognized, especially in developing countries where the majority of citizens depend on agriculture as a source of livelihood. If rural development is to be sustainable, the rural youth need to be brought in the mainstream of the development process, no matter whether the development initiatives come from the public or private sector. Rural development in the long-term depends on how the youth are prepared to cope with the challenges they are likely to face as rural citizens.

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Keywords: Youth, Rural development, Employment, Migration

1. Introduction

There is a widespread belief that rural youths have vital role to agricultural and rural development (Malatest, 2002; Odhiambo, 2001; Gameren and Hinojosa, nd).The identify factors affecting on migration youths to urban centers is very important to rural program development. For develop strategies that attract and keep youth in rural communities, reasons youth migrate to urban centers must be closely examined and identified. According to survey results, rural youth who currently live or have lived in urban communities relocated to large centers to pursue post-secondary education, find employment, or accompany family (Malatest, 2002). According to Fajans et al (nd), rural youth have less opportunities in period of after formal education programming and activities than their urban counterparts. It is also acknowledged that youth get into trouble more often if they have nowhere to go and no meaningful activities to keep them occupied in after school hours. Although youth migration has been a popular subject in recent years, little research has focused on the migration between rural and urban areas. One of the main factors affecting young people's success to employment in agricultural practices was their connection with local informal networks. Informal networks provided young people with information about forthcoming employment opportunities or personal recommendations for jobs. (Cartmel and Furlong, 2000). Although youth migration has been a popular subject in recent years, little research has focused on the migration between rural and urban areas. According Ommani and Chizari (2006) Extension educators can extend the opportunities in youth development issues. They have the ability to help youth develop in different ways. In addition, efforts should be made to help youth develop the capacity to voice their needs within the cultural, social, and political framework. FAO (1999) explained" currently, 472 million young people are living in rural communities in developing countries. Unfortunately, many rural young people are choosing instead to migrate to the city in order to make a better life for themselves or send money home to help their families – an exodus that to constitutes a severe threat to global food security"(p. 17).

Agriculture is a vital sector of the economy of Iran. Currently, about one-fourth of the nation's Gross National Product, one-third of the work force, more than four-fifths of the nation's food needs, one-

third of non-oil exports, and nine-tenths of industry is dependent on agriculture (Ommani, 2006). Rural youth of Iran have vital role to agriculture sector, especially in livestock husbandry. Among the activities of the agriculture sector, livestock husbandry has an important and special role. It not only provides the protein needs of the people, but eighty-five percent of the work force in the agriculture sector is involved, full or part-time, in livestock production. A greater understanding of this sector is needed as Iran addresses its goal of self-sufficiency in the production of food and fiber products (Chizari, Bahmani, & Lindner, 2001; Chizari, Lindner, & Lashkarara, 2001; Ommani, 2006). In this paper, we determined the strategies for retaining youth to rural communities.

Objectives

The specific objectives of this study were to:

1) Describe rural youths in Shoushtar township of Khuzestan province by demographic characteristics.

2) Describe reasons for moving to urban centers cited by rural youth.

3) Identify strategies for retaining youth to rural communities.

2. Material and Methods

The research design was a descriptive method. The population consisted of 360 rural youths of Shoushtar Township, Khuzestan Province of Iran (Krejcie and Morgan, 1970). From a review of the literature, the researchers developed an instrument to collect data (Samardick, 2000; Malatest, 2002; Cartmel & Furlong, 2000; Odhiambo, 2001; Valentine et al, 2005). Face and content validity of the questionnaire was established using a panel of experts consisting of faculty in the Department of Agricultural at Islamic Azad University- Shoushtar Branch, Iran. A pilot test was conducted with 15 youths. Questionnaire reliability was estimated by calculating Cronbach's alpha. Reliability for the overall instrument was .83. Data were collected through a structured interview and a questionnaire with youths at their rural. The response rate was 97%. According to Lindner, Murphy, and Briers (2001), nonresponse error is not a threat to external validity of a study when an 85% response rate is achieved. Data collected were analyzed using the Statistical Package for the Social Sciences (SPSS). Appropriate statistical procedures for description (frequencies, percent, means, and standard deviations) were used.

3. Results

The following section present finding by objective:

Objective1

The first objective was to describe rural youths in Shoushtar township of Khuzestan province by demographic characteristics. All respondent were male. Approximately 45% of respondents between 18 to 21 year. Rural youths were asked to report their highest level of education: 34% of youths had an elementary education; 16% were illiterate; 35% had high school diploma; 15% had post high school education.

Objective 2

The second objective was assessing top reasons for moving to urban centre. According to survey results, top reasons by youth idea that living in rural including: employment, education, family-related and to get away (Chart 1).

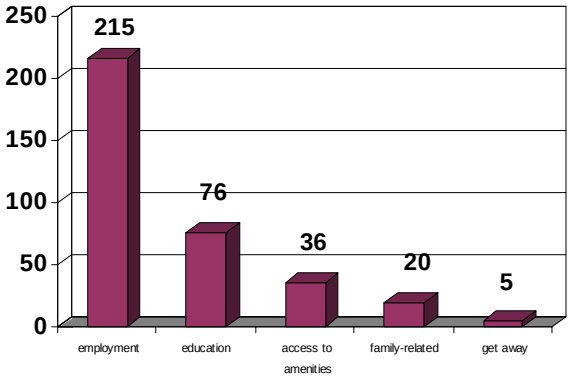

Chart 1. Top reasons for moving to urban centers.

Objective 3

The third objective was to identify strategies for retaining youth to rural communities. The top eight strategies for retaining youth to rural communities were (Table 1): Improve career opportunities**,** Provide work experience opportunities **,** Improve opportunities for education after high school**,** Improve opportunities for social activities**,** Improve access to amenities**,** Promote the advantages of rural living**,** establishment of youth advisory committees establishment of youth priorities for local government**,** Promote youth involvement in community decision making.

According to the correlation analysis, there was a significant relationship between crop yield, income, land ownership, and mechanization with level of perception of rural youth to rural living were significantly positive.

Table 1. Strategies for retaining youth to rural communities**.**

Reason	Mean	SD	Rank
Improve career opportunities	4.25*	.78	1
Provide work experience opportunities	3.95	.75	2
Improve opportunities for education after high school	3.68	.89	3
Improve opportunities for social activities	3.46	.85	4
Improve access to amenities	3.24	1.03	5
Promote the advantages of rural living	3.13	1.13	6
establishment of youth advisory committees	2.90	1.23	7
establishment of youth priorities for local government	2.71	1.13	8
Promote youth involvement in community decision making	2.73	1.29	9

*1)Not important; 2)Little important; 3) Somewhat important; 4)Very important; 5) Extremely important

Table2. Correlation between some characteristics with perception of rural youth to rural living

Characteristics	**r**	**p**
Level of education	.015	0.652
Land ownership	0.606	0.000***
Income	0.754	0.000***
Social participation	0.214	0.003**
Social status	0.013	0.618
Mechanization level	0.554	0.000***
Use of communication channel	0.115	0.050*
Crop yield	0.587	0.000***

Note. *: p<0.05; **: p<0.01; ***: p<0.001

Table4**.** Liner regression for predict changes in perception of rural youth to rural living

Variable	**B**	**SE B**	**Beta**	**T**	**Tsig**
Income (x_1)	0.325	0.635	0.875	3.351	0.004
Social participation (x_2)	0.553	0.444	0.234	4.442	0.000
Mechanization level (x_3)	0.769	0.236	0.344	5.436	0.000
Perception to agricultural practices (x_4)	0.986	0.275	0.556	5.339	0.000
Crop yield (x_5)	0.556	0.625	0.245	4.356	0.000
Signif F =0.000			F= 81.123		
R^2=0.760			R= 0.871		

In continue used liner regression for predict changes in perception of rural youth to rural living. Income, Social participation, Mechanization level, Perception of rural youths' awareness with respect to agricultural practices and Crop yield may well explain for 76% changes (R^2=.76) in perception of rural youth to rural living.. This relationship is described in the following formula**:**

$Y=4.563+0.325\ x_1+0.553\ x_2+0.769\ x_3+0.986\ x_4+0.556\ x_5$

4. Recommendations and Implications

From a development perspective, the youth are the future for any country and the world. The potential of youth to transform rural communities needs to be recognized, especially in developing countries where the majority of citizens depend on agriculture as a source of livelihood. If rural development is to be sustainable, the rural youth need to be brought in the mainstream of the development process, no matter whether the development initiatives come from the public or private sector. Rural development in the long-term depends on how the youth are prepared to cope with the challenges they are likely to face as rural citizens.

The research confirmed that while much of this outflow could be attributed to employment, educational and social factors, there are a number of activities or actions that could be implemented to help redress the factors that contribute to rural youth migration. The research also confirms that for many rural youth, the relocation to a larger urban centre is both an economic and social priority. However, many of these same youth would return to a rural or small

town community if such communities could be made more attractive to youth.

Extension educators can extend the opportunities in youth development issues. They have the ability to help youth develop in different ways. In addition, efforts should be made to help youth develop the capacity to voice their needs within the cultural, social, and political framework. We must :

•Provide a conditions for rural youth to connect with peers across the state and share information to rural youth.

•Effort to communicating and exchanging information, including establishing the teamwork in youth club.

•Identify that rural youth have limited access to post-secondary education opportunities; it is important that education and training institutions provide sufficient opportunities for rural youth to acquire the skills and knowledge, particularly those that could be valuable to the local community.

The results also showed that top four strategies that could be implemented by organizations to support the economic and social conditions conducive to increasing the desire of rural youth to remain in and/or return to rural communities were:1) Improve career opportunities, 2) Provide work experience opportunities, 3) Improve opportunities for education after high school, and 4) Improve opportunities for social activities.

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Melatonin Supplementation Could Trigger Delayed Cardiac Preconditioning Against I/R Injury in Partial Nephrectomized Rats with Emphasis to Possible Role of Cardiac NO.

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Abstract: The cardioprotective effects of melatonin are consistent with its ability to scavenge free radical. However free radicals are considered as preconditioning factors, helping the heart to withstand consequent attacks of ischemic reperfusion injury. So, this study aimed to clarify whether melatonin supplementation, concomitant with the deterioration of kidney function in experimental model of renal failure, is able to protect the isolated heart against the liability for global ischemic reperfusion (I/R) injury or its antioxidant effect interferes with proposed preconditioning effect of free radical. Moreover, the study evaluated the changes of myocardial nitric oxide (NO) system with melatonin treatment as one of the suggested triggers of preconditioning. Thirty male Albino rats were divided into three equal groups, sham- operated control rats, 5/6 subtotal nephrectomized (STNx) group and 5/6 subtotal nephrectomized melatonin- supplemented (STNx + M) group. Melatonin was given at a dose of 5 mg / kg/ day for 8 weeks. Rats in all groups were subjected to estimation of plasma urea, creatinine, malondialdehyde (MDA) and nitrate levels, followed by perfusion of isolated hearts. A period of ischemia (30 min) followed by reperfusion for another 30 min was done. The cardiac hemodynamic changes during reperfusion at 5, 15, 25 and 30 min intervals were recorded. At the end of reperfusion, the different chambers of the heart were subjected for determination of the absolute weights as well as their weights to body weight ratios. Sections from the cardiac muscle, mainly ventricle, were used for tissue reduced glutathione (GSH) and nitrate estimation. Partial nephrectomized group (STNx) exhibited significant deterioration of the baseline cardiac hemodynamic as well as more liability for ischemic reperfusion injury in early (5 min) and late reperfusion (30 min) records. Also nephrectomy caused significant cardiac remodeling (hypertrophy), manifested in the increased left ventricle and whole cardiac weights to body weight ratio. The significantly increased plasma MDA, urea and creatinine with nephrectomy showed a negative correlation with the reduced plasma and cardiac tissue nitrate. Melatonin treatment concomitant with the deterioration of renal function(in STNx +M group) showed significant higher basal coronary flow compared to STNx group but it did not improve the ameliorate basal intrinsic cardiac activity due to renal failure. Following I/R, melatonin pre treated group showed some sort of protection against deterioration of cardiac activity in particular at 30 min reperfusion. A 44.5 % decrease in HR in STNx rats versus 30.5% decrease in HR in melatonin treated has been observed. Also the percentage of decrease in peak tension and the tension /left ventricular weight due to reperfusion were significantly lower with melatonin treatment at both 5 and 30 min records of reperfusion. Also melatonin shortened the time to peak tension (TPT) in particular at 30 min reperfusion where ,the increase in TPT due to reperfusion injury was +20.3% with melatonin treatment versus +51.9% in non treated rats. Although, melatonin shortened the half relaxation time(1/2RT) and improve the myocardial flow rate(MFR) compared to non treated group in some records of reperfusion but compared to basal record ; the percentage of change was non significant. Melatonin significantly decreased urea, creatinine and MDA levels which still higher compared to sham control group. Also melatonin ameliorated the hypertrophic changes but not completely with an increase in cardiac tissue GSH and nitrate levels in hearts of melatonin treated rats as well as plasma nitrate.
The increased MDA which is an indicator for free radical generation in partial nephrectomized rats did not provide the supposed preconditioning effect against ischemic reperfusion injury in isolated hearts or its effect wasn't conclusive. On the other hand, melatonin was able to improve the basal coronary flow rate and appears to offer some sort of preconditioning and/or protection against I/R injury a condition of excess free radical generation. Cardiac tissue GSH (anti-oxidant) and NO triggering by melatonin may be added to its free radical scavenging effect in the suggested protection and / or preconditioning.

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Key words: cardiac preconditioning, ischemic reperfusion, melatonin, nitric oxide, free radicals, partial nephrectomy.

1. Introduction:

Nowadays, cardiovascular diseases represent the most important health risks as they are responsible for more than 50% of total mortality. Among them, ischemic heart disease is the leading cause of morbidity and mortality (Ostadal 2009). In coronary patients aging, diabetes and other factors interfere with most of cardioprotectants as well as with preconditioning-based interventions (Downey and Cohen 2009). Similarly renal diseases are commonly associated with accelerated cardiovascular disease such as ischemia, pericarditis and peripheral vascular disease due to uremic toxins, and electrolyte disturbances(Leineweber *et al.,* 2002), dyslipidemia, endothelial dysfunction, oxidative stress, and inflammation(Schiffrin *et al.,* 2007)

Ischemic-reperfusion injury to cardiac myocytes is mediated by an overproduction of oxygen free radicals in part during ischemia and more abundantly during early reperfusion (Petrosillo *et al.,* 2006)and chronic renal failure is commonly associated with increased free radical generation (Schiffrin *et al.,* 2007), thereafter, appropriate antioxidant strategies could be considerably useful in protection against cardiac ischemic reperfusion injury(I/R) in renal failure.

Melatonin is considerably better than the classic antioxidants in resisting free-radical–based molecular destruction as it is mentioned in several studies to be a suicidal or terminal antioxidant which sacrifices itself and does not participate in redox cycling after scavenging free radicals (Korkmaz *et al.,* 2009*).* Moreover, melatonin has a well known cardioprotective effect in view of its free radical-scavenging activity or through its direct interaction with its cardiovascular receptors (Paulis & Simko 2007). Some studies used melatonin before ischemia or during reperfusion *(in vitro)* (Szarszoi *et al.,* 2001) and others used it, *in vivo,* just before ischemia (Sahna *et al.,* 2005).

Free radicals has been claimed to play an important role in the triggering action of ischemic preconditioning , where, exposure of the heart to one or more short episodes of ischemia protects against subsequent long period of ischemia (Genade *et al.,* 2006 and Bolli 2007). Preconditioning protects the myocytes as well as the endothelium of large epicardial and intra myocardial coronary arteries against reperfusion injury. The short ischemic attack cause a transient increase in reactive oxygen species(ROS) which suggested to activate protein kinase C causing reduction in endothelial-nurophil interaction and /or activation of gene expression of protective proteins such as NO synthases and antioxidant enzymes (Laude *e tal.,* 2002). Moreover, ROS suggested reducing pore opening in inner mitochondrial membrane resulting in reduction in ROS production by mitochondria during reperfusion (Matsuzaki *et al.,* 2009). However, Preconditioning is used now as generic term to encompass any protocol applied before prolonged ischemia that protect the heart during ischemic reperfusion (Halestrap *et al.,* 2007)

Nitric oxide (NO), an important cellular signaling molecular or chemical mediator, beside its well known formation by endothelium, it is formed by the coronary endothelium, endocardial endothelium, cardiac nerves and cardiomyocytes of the normal heart as all express NO synthase and have basal production of nitric oxide (Zweier & Talukder 2006). NO has been shown to play an important role in the myocardial preservation via its vasodilator, antioxidant, anti platelet and anti-neutrophil actions (Schulz *et al.,* 2004). Also it has been confirmed that NO mediates the ischemic preconditioning and many drug can alleviate myocardial ischemia-reperfusion injury by promoting NO production (Cuong *et al.,* 2006),

Thereafter, antioxidants may block the preconditioning effects of free radical and so the liability for ischemic reperfusion injury may be increased .The aim of this study was, therefore, to establish whether melatonin, in view of its free radical-scavenging has a cardioprotective effect or it interferes with the supposed preconditioning effects of free radical. Also the contribution of NO to the possible effect of melatonin is considered during this investigation.

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2-Materials and Methods:

2.1-Experimental Animals:

This study was carried out on male thirty albino rats, weighing 180-200gm. They were purchased from military animal farm (Cairo) and maintaining in our hold facilities in the Physiology Department under standard conditions of boarding and given ordinary rat diet. Water was available ad libitum.

2.2-Experimental protocol:

Experimental animals were allocated into three equal groups as follows:

Group I (10 rats): Sham- operated control rats.

Group II (10 rats): Two stages subtotally nephrectomized rats (STNx) without melatonin treatment.

Group III(10 rats):Two stages STNx rats treated with melatonin (Amon Co.), at a dose of 5 mg / kg/day by oral gavages for 8 weeks, immediately started after the second stage of the operation

2.3-Experimental procedure:

Subtotal nephrectomy was conducted according to Hatori *et al.* (2000), where anaesthetized rats using Ether were laparotomized and 2/3 of the left kidney was removed. 4-5 days later the labarotomy was repeated and the right kidney was totally removed resulting in 5/6 subtotal nephrectomy. The rats had restricted to water and standard rat chaw, food consumption was checked twice a week.

Melatonin supplementation:

Rats were supplemented by Melatonin in a dose of 5 mg /kg/day according to (Lee *et al.,* 2002; Stacchiotti1 *et al.,* 2006). Melatonin was obtained as a powder supplied by Amon Co. Egypt. Melatonin powder was dissolved in distilled water as 5mg/10 ml and each rat received the calculated dose according to its weight by gavages. Sham control rats and STNx rats received distilled water by gavages instead of melatonin.

Blood sampling:

Eight weeks after the second stage of the operation and after overnight fasting, rats were weighed, injected with 1000 IU heparin sodium half an hour before anesthesia. Animals were anaesthetized by intraperitoneal injection of sodium thiopental in a dose of 40mg /kg B.W (Nile CO.). Labarotomy was done and the abdominal aorta was cannulated and the blood samples were collected in heparinized tube for determination of the levels of plasma creatinine, urea, MDA and nitrate.

Isolated heart ischemic –reperfusion:

Rapidly after blood sampling, the chest of the rat was opened and the heart was excised. The heart was immediately chilled in ice –cold modified Krebs-Henseleit bicarbonate buffer solution (pH 7.4) for fast cardioplegia and to prevent ischemia. The aorta was then cannulated and a retrograde perfusion with Krebs- Henseleit bicarbonate buffer solution was started under constant pressure (55 mmHg) without recirculation. The solution gassed with 95% O2 and 5% CO2, according to modified Langerdorff technique described elsewhere by Ayobe and Tarazi (1983) The entire system was jacketed in water to maintain a temperature of 37 C. The tension by the heart was measured by a light weight (0-50g) range D1-isometric force transducer which is connected through a strain gauge coupler FC117 to a two channel oscillograph (Washington MD2 Bioscience). One gram weight was attached to the heart apex and was left to hang freely. After 15 minutes (stabilization period), the base line activities were recorded at 50 mm/sec paper speed. Total global ischemia was induced by stopping delivering of the perfusion fluid for 30 minutes. Afterwards, the hearts were reperfused again for additional 30 minutes. The duration of ischemic reperfusion injury selected according to Nakamura *et al.,* (1991)

Hemodynamic parameters:

In each record, Heart rate (HR, beat/min), peak developed tension (PT, gram), time to peak tension (TPT, msec.), half relaxation time (½ RT, msec.) and myocardial flow rate
(MFR, ml /min) were determined at different intervals of reperfusion, at 5, 15, 25 and 30 minutes. In addition, basal peak tension / LVW (g/ 100mg) and basal myocardial flow rate / LVW (ml/min/100mg) was calculated later on.

Cardiac tissue handling:

At the end of ischemic reperfusion procedure, hearts were thoroughly cleaned from fat and fibrous tissues. The atria were separated, the right ventricular wall peeled evenly and remaining left ventricle +septum were all blotted dry using absorbing paper and weighed in a 5-digit –Metler balance (AP 160). Cardiac weights of atria, right ventricle, left ventricle, and whole heart were expressed as absolute, as well as relative weights normalized to body weight (mg/g).

After weighing, the cardiac tissue of the left ventricle was homogenized according to Eissa *et al.,* (1990) in homogenization buffer (PH 7.2); for each 0.1mg cardiac tissue, 1ml buffer is added. The buffer consisted of (0.32 mmol/l Sucrose, 20 mmol /l N-2 hydroxyethyl piperzine N-2 ethansulfonic acid (HEPES).,0.5 mmol/l Ethylene diamine tetra – acetic acid (EDTA).,1 mmol /l Di Thiotheritol (DTT),1 mmol/l Phenyl methane sufonyl floride (PMSF) (Sigma).After homogenization, the homogenate was cooled for 10 min. in an iced water bath; then the samples were centrifuged for 10 min. at 4000 rpm. The supernatant was stored in aliquots at _ 80C for subsequent estimation of cardiac tissue nitrate and GSH.

Biochemical analysis:

Measuring plasma urea was formed according to the method described by Searcy *et al.,* (1967), by using kits supplied by Biolabo, France, and depending on colorimetric method, adjusted at wave length 600 nm.

Concentration of creatinine in plasma was estimated according to Jaffe reaction described by Fabiny and Ertingshausen (1971) and Labbe *et al.,* (1996) by using kits supplied by Biolabo, France, depending on colorimetric method adjusted at wave length 490 nm.

Plasma MDA level (nmol /L) was determined as an indicator of lipid peroxidation products in plasma. The

principle of the method is the spectrophotometric measurement of the color generated by the reaction of thiobarbituric acid (TBA) with MDA, according to the technique of Esterbauer & Cheesman (1990). The concentrations were then divided by 1000 to be expressed in µmoles.

Nitrate assay: Plasma samples, as well as, supernatant of cardiac tissue homogenate were subjected to nitrite assay using reagents supplied by Sigma. nitrate used as an indicator for nitric oxide level since nitric oxide is extremely unstable lipid-soluble gas and undergoes rapid oxidative degradation to the stable breakdown products nitrate and nitrite. nitrate determined according to a method described by Bories and Bories (1995). The concomitant reduction of nitrate to nitrite by NADPH was monitored by the oxidation of the coenzyme and the decrease in the absorbance at 340 nm. Plasma nitrate was expressed as µmol/l, whereas cardiac tissue nitrate levels were expressed as µmol/g.

Cardiac tissue GSH:

Supernatant of cardiac tissue was also subjected for estimation of GSH level. GSH was determined by the spectrophotometric method according to method of Jollow *et al.,*(1974). The kits provided by Bio-diagnostic and the results were expressed as nmol/g tissue.

Statistical Analysis:

Results were expressed as mean ± SEM. The statistical significance of differences between means was determined by student's 't' test for both paired and unpaired group at a level of significance $p < 0.05$.. Data was analyzed by ANOVA, Statistical analysis and correlations were calculated by Pearson correlation (2-tailed) using SPSS program (statistical progression for social science) statistical package (SPSS Inc.) version 8.

Percentage of changes was calculated in all the studied groups, at 5 minute and 30 minute during reperfusion relative to the basal record.

3-Results:

As shown in table (1); urea and creatinine levels were significantly higher ($p<0.05$) in partial nephrectomized group (STNx) compared to sham control group,. The plasma level of MDA was significantly ($p<0.05$) increased, while plasma nitrate concentration was significantly ($p<0.0$) decreased. The cardiac tissue levels of both GSH and nitrate were significantly decreased. Also, the body weight was significantly decreased (% of change from initial weight was 46.43% in STNx rats compared to 71.55 % in sham control).

Melatonin treated group (STNx+M) showed significant ($p<0.05$) lower urea and creatinin level compared to the non treated nephrectomized rats but their levels still significantly ($p<0.05$) higher compared to sham control rats. Melatonin also significantly ($p<0.05$) lowered the plasma MDA level and significantly ($p<0.05$) increased plasma nitrate concentration compared to STNx rats. In addition, melatonin treatment caused significant ($p<0.05$) increase in cardiac tissue levels of both GSH and nitrate. The body weight in the melatonin treated group (STNx+M) was significantly ($p<0.05$) higher compared to nephrectomized non treated rats (STNx), but it still significantly ($p<0.05$) lower compared to sham control rats.

Table (1): showing the changes in plasma Urea, Creatinine , nitrate and MDA and cardiac tissue levels of GSH and nitrate as well as the % of change in body weights(gm) in the different studied groups

	Sham control (n=10) Mean ± S.E.M	**STNx (n=10)** Mean ± S.E.M	**STNx+M(n=10)** Mean ± S.E.M
Urea(mg/dl)	29.5 ±0.36	82.5 ± 0.67**a**	64.7 ± 2.16**ab**
Creatinine(mg/dl)	0.29 ± 0.01	0.99 ± 0.02**a**	0.49 ± 0.02**ab**
Plasma nitrate(µmol/L)	14.5 ± 1.14	5.83 ± 0.6**a**	9.48 ±0.84**ab**
Plasma MDA(µmol/L)	1.76± 0.09	2.77 ± 0.15**a**	2.15 ±0.05**ab**
Cardiac tissue GSH. (nmol/g)	465.3 ±6.16	302.0 ± 8.7**a**	410.0 ±11.9**ab**
Cardiac tissue nitrate. (µmol/g).	894.0 ± 5.1	704.6±5.6**a**	808.1± 5.5**ab**
Final B.W	327.5± 5.1	281.0± 4.6**a**	311.5 ± 3.2**ab**
BW (% of change)	71.55%	46.43%	62.8%

Changes in cardiac weights:

As shown in table (2) and figure (1); in the STNx group, the ratios of heart weight and the left ventricle (including septum) to body weight as well as the left ventricle to right ventricle weight ratio ,all were increased significantly ($P<0.001$ for all) compared with those of sham control.

In melatonin treated -nephrectomized group (STNx +M), the relative weight of whole heart and left ventricle exhibited highly significant (P< 0.001) decrease compared to the nephrectomized group but they still having significantly (p<0.05) higher values compared to sham control. Melatonin also significantly (p<0.05) decreased the LV/ RV ratio compared to nephrectomized rats.

Table (2) Changes in absolute cardiac weights and cardiac weight body weight ratios in control, nephrectomized(STNx) and nephrectomized melatonin- treated rats(STNx + M).

Cardiac weight	Sham controls (n=10) Mean ± SEM	STNx(n=10) Mean ± SEM	STNx +M(n=10) Mean ± SEM
Atrium (At, mg)	53.8 ± 1.19	44.97 ± 2.11[a]	48.02 ± 1.56[a]
At / BW(mg/g)	0.16 ± 0.002	0.16 ± 0.00	0.16 ± 0.005
Right ventricle(RV. mg)	78.9 ± 2.08	68.18 ± 2.21[a]	71.86 ± 1.4[a]
RV/ BW (mg/ g)	0.24 ± 0.004	0.24 ± 0.007	0.23 ± 0.004
Left ventricle(LV. mg)	685.3 ± 18.11	718.5 ± 15.6	711.0 ± 13.22
LV/BW (mg/g)	2.09 ± 0.04	2.58 ± 0.03[a]	2.28 ± 0.05[ab]
LV/RV (mg/mg)	8.72 ± 0.23	10.69 ± 0.32[a]	9.92 ± 0.21[ab]
Whole heart(WH. mg)	818.0 ±20.03	837.15 ± 17.43	830.9 ± 14 .17
WH/BW(mg/g)	2.50 ± 0.04	2.98 ± 0.03[a]	2.67 ± 0.05[ab]

a: Significance by LSD at P <0.05 relative to control group.
b: Significance by LSD at P <0.05 relative to STNx group(subtotal nephrectomy).

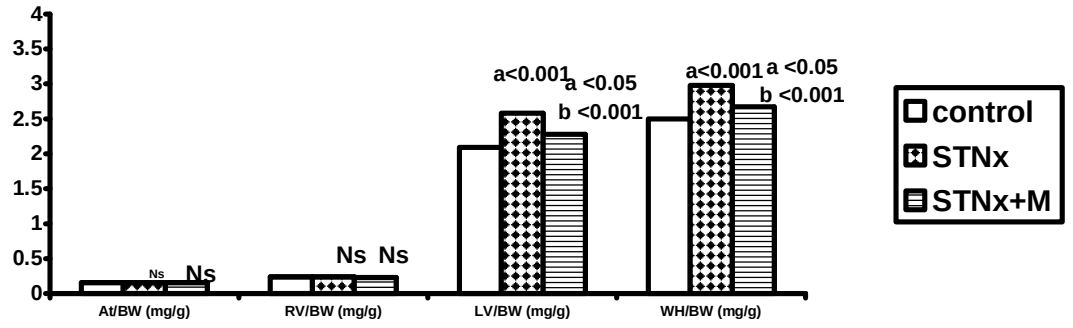

Fig. 1:- Changes in cardiac weight - body weight ratios in the different studied groups

Base line cardiac performance:

As shown in table (3); in STNx rats, the basally recorded *in vitro* cardiac activities were clearly altered. This was manifested in the significantly decreased, heart rate, peak developed tension and myocardial flow rate compared to their sham control rats. The calculated basal, myocardial flow rat per 100 mg LVW as well as peak tension per 100mg LVW was also significantly reduced compared to sham control rats. Moreover, the basal time to peak tension and half relaxation time were significantly prolonged in hearts of STNx rats. Melatonin treatment (in STNx+ M) was able to increase significantly (p<0.05) the myocardial flow rate while the myocardial flow rate per/100mg LVW was border line
Significantly (P≤ 0.05) increased.

On the other hand, in melatonin treated-nephrectomized rats (STNx+M) ,the values of basal heart rate , peak developed tension , tension /100mg LVW, time to peak tension and half relaxation time did not significantly differ from that observed in non treated nephrectomized rats.

Table (3): Showing basal activity of intrinsic cardiac properties in the different studied groups.

	Sham control Mean ± SEM	**STNx** Mean ± SEM	**STNx +M** Mean ± SEM
HR (beat/ min) (n=10)	187.4 ±10.1	146.5 ±7.2**a**	163.0±6.5[a]
Peak developed tension (PT, gram) (n=10)	6.2 ± 0.51	4.6 ± 0.29**a**	4.6 ± 0.26 **a**
Peak tension/ LVW (g/100mg) (n=10)	0.91± 0.08	0.64± 0.04[a]	0.65 ± 0.04[a]
Time to peak tension (TPT, msec) (n=10)	88.0 ± 7.4	140.0± 7.2**a**	137.0± 8.9**a**
Half relaxation time (HRT, msec) (n=10)	82.0 ± 7.4	176.0 ± 7.6**a**	156.0± 8.2**a**
Myocardial flow rate (MFR, ml/min) (n=10)	7.5± 0.37	4.41± 0.47**a**	5.6 ± 0.39**ab**
MFR / LVW (Ml/min/100mg) (n=10)	1.06± 0.05	0.61± 0.07**a**	0.79± 0.06**ab**

a: Significance by LSD at P <0.05 relative to sham control group.
b: Significance by LSD at P <0.05 relative to STNx group

Ischemic reperfusion results:

As shown in tables (4, 5 & 6); compared to pre-ischemic basal level of cardiac activity, the three studied groups exhibited nearly the same pattern of response to I/R with different intensities. Significant($p<0.05$) decrease in beating rate (HR), depression in peak tension (PT) and tension/100mg LVW, diminished myocardial flow rate (MFR) and flow rate / 100mg LVW, as well as significantly ($p<0.05$)prolonged time to peak tension (TPT) and half relaxation time (HRT) have been observed.

Chronotropic activity:

As shown in table (4-A); in STNx group during reperfusion of the isolated hearts, significant($P<0.05$) decrease in heart rate was observed compared to the corresponding records in sham control(at 15,25&30 minute) and compared to its basal records($P<0.0005$). Melatonin treatment in STNx+M group increased the HR compared to non treated group, the increase was significant ($P<0.05$) at 25 and 30 minute reperfusion records.

Peak developed tension and tension per LVW:

As shown in table (4-B & C); in STNx group the peak developed tension as well as the tension per 100mg left ventricular weight were significantly($P<0.05$) reduced in all records of reperfusion when compared to the corresponding records in sham control rats and when compared to their basal records($P<0.0005$ or $P<0.001$). In melatonin treated rats, compared to STNx rats, the tension only increased significantly ($P<0.05$) at 5 min. reperfusion record. Also the tension / 100mg LVW was significantly ($P<0.05$) increased only at 5and 30 min.

Table (4): Shows the pre-ischemic (basal) records and the changes in heart rate (HR), peak developed tension (PT) and peak tension/ LVW (g/100mg) at different intervals of reperfusion in the different studied groups.

A- Heart rate

Parameters	**Group**	**Basal**	**5min.**	**15min.**	**25min.**	**30min.**
HR (Beat/min)	Sham control Mean ± SEM N=10	187.4 ±10.1	125.0 ± 9.9**c**	136.0 ± 11.9**c**	128.0±10.3**c**	119.0± 11.0**c**
	STNx Mean ± SEM N=10	146.5 ±7.2**a**	102.6 ±7.6**c**	98.5 ± 6.9**ac**	81.0 ± 6.2**ac**	80.5 ± 5.9**ac**
	STNx+M Mean ± SEM N=10	163.0±6.5	129.5±11.7**c**	121.0± 7.8**c**	112.5±7.8**bc**	113.5± 8.4**bc**

B- Peak tension

Parameters	**Group**	**Basal**	**5min.**	**15min.**	**25min.**	**30min.**
PT (gram)	Sham control Mean ± SEM N=10	6.2 ± 0.51	4.12 ± 0.33**C**	3.85 ± 0.29**C**	3.89 ± 0.26**C**	3.65± 0.2**C**
	STNx Mean ± SEM N=10	4.6 ± 0.29**a**	2.98 ± 0.22**ac**	3.15 ± 0.20**ac**	2.85 ± 0.23**ac**	2.7± 0.3**ac**
	STNx+M Mean ± SEM N=10	4.6 ± 0.26	3.8 ± 0.22**b**	3.3 ± 0.20**c**	3.1 ± 0.17**c**	3.04 ± 0.2**c**

C-Peak tension /LVW

Parameters	Group	Basal	5min.	15min.	25min.	30min.
PT/ LVW gm/100mg	Sham control Mean ± SEM N=10	0.91± 0.08	0.6 ± 0.05[c] <0.0005	0.56 ± 0.04[c] <0.0005	0.57± 0.04[c] <0.0005	0.54± 0.04[c] <0.0005
	STNx Mean ± SEM N=10	0.64± 0.04[a]	0.41 ±0.03[ac]	0.43 ± 0.03[ac]	0.40 ± 0.03[ac]	0.38 ± 0.04[ac]
	STNx+M Mean ± SEM N=10	0.65 ± 0.04	0.53± 0.03[bc]	0.46± 0.03[c]	0.43± 0.03[c]	0.48± 0.02[b]

a: Significance by LSD at P <0.05 relative to sham control group. **b:** Significance by LSD at P <0.05 relative to STNx group.
c: Significance at each record, 5, 15, 25&30 minutes of reperfusion relative to baselineValues.

Time to peak tension:

As shown in table (5); changes in time to peak tension (TPT) during reperfusion showed that nephrectomized rats exhibited significant (P<0.05) prolonged TPT throughout all records of reperfusion compared to sham control. Also they demonstrated significant longer TPT compared to their TPT basal records (P<0.02, P<0.005 &P<0.0005 at 5,15 &both 25 and 30 min. respectively). Melatonin treatment did not alter the TPT significantly except for at 30 min. reperfusion record where it was significantly (P<0.05) shorter compared to nephrectomized group.

Half relaxation time:

As shown in table (5); STNx rats showed significantly prolonged half relaxation time (HRT) in all records of reperfusion compared to sham control (P<0.05), as well as compared to their basal record (P<0.01,P<0.002,P<0.005&P<0.004 at 5,15,25 and 30 min. records respectively). In the Melatonin treated rats (STNx+M), the HRT was significantly (p<0.05) shorter compared to STNx rats at 5 and 30 minutes reperfusion and shorter though none significantly at 15 and 25 min. reperfusion.

Table (5) Shows the pre-ischemic (basal) records and the changes in time to peak tension (TPT) and half relaxation time (1/2 RT) at different intervals of reperfusion in the different studied groups.

Parameters	Group	Basal	5min.	15min.	25min.	30min.
TPT (msec.)	Sham control Mean ± SEM N=10	88.0 ± 7.4	110.0± 12.9	113.0 ± 9.32[c]	119.0 ± 7.1[c]	120.0 ± 7.6[c]
	STNx Mean ± SEM N=10	140.0± 7.2[a]	162.0 ±9.97[ac]	173.0 ±8.6[ac]	179.0 ± 5.0[ac]	210.0 ± 11.5[ac]
	STNx+M Mean ± SEM N=10	137.0± 8.9	169.0 ± 16.1[c]	164.0 ± 8.6[c]	165.0 ± 10.0[c]	163.0 ± 10.2[cb]
HRT (msec.)	Sham control Mean ± SEM N=10	82.0 ± 7.4	97.0 ± 6.8[c]	107.0± 8.0[c]	116.0± 7.8[c]	110.0 ± 7.6[c]
	STNx Mean ± SEM N=10	176.0 ± 7.6[a]	202.0 ±11.4[ac]	209.0 ± 10.9[ac]	215.0 ±14.5[ac]	217.0 ±13.5[ac]
	STNx+M Mean ± SEM N=10	156 ± 8.2	172.0 ± 7.9b[c]	186.0 ± 9.4[c]	185.0 ± 11.2[c]	185.0 ±11.24[bc]

Myocardial flow rate:

As shown in table (6); the myocardial flow rate and myocardial flow rate /100mg LVW were significantly(P<0.05) reduced during all reperfusion records following ischemia in nephrectomized rats compared to sham control and compared to their basal record. Melatonin treatment increased the MFR and the corrected MFR significantly (P<O.05 for both) at

both 5 and 30min reperfusion record compared to nephrectomized non treated rats.

As shown in table (7); in STNx group, the % of change did not differ significantly compared to the corresponding % of change in sham control rats in almost all of the studied parameters except for the reduced myocardial flow rate and myocardial flow rate per left ventricular weight in STNx rats. The MFR significantly (P<0.05) decreased by – 14.1 ± 3.11 % in sham control, verss – 26.9 ± 3.1 in STNx. Also, the MFR/LVW significantly (p<0.05) decreased by – 11.7 ± 2.5 % in sham control versus –26.8±3.4 in STNx group.

Table (6): Shows the pre-ischemic (basal) records and the changes in myocardial flow rate (MFR) and myocardial flow rate / LVW, at different intervals of reperfusion in the different studied groups.

parameters	Group	Basal	5min.	15min.	25min.	30min.
MFR (ml /min.)	Sham control Mean ± SEM N=10	7.5± 0.37	6.45 ± 0.46^{c}	5.9 ± 0.44^{c}	5.2 ± 0.4^{c}	5.15± 0.43^{c}
	STNx Mean ± SEM N=10	4.41± 0.47^{a}	3.2 ± 0.31^{ac}	3.10 ± 0.24^{ac}	2.8 ± 0.22^{ac}	2.7 ± 0.22^{ac}
	STNx+M Mean ± SEM N=10	5.6 ± 0.39^{b}	4.2 ± 0.28b^{c}	3.97 ± 0.29^{c}	3.5 ±0.35^{c}	3.96 ± 0.29^{bc}
MFR/ LVW (ml /min/ 100gm)	Sham control Mean ± SEM N=10	1.06± 0.05	0.94 ± 0.07^{c}	0.86± 0.06^{c}	0.76± 0.06^{c}	0.75 ± 0.06^{c}
	STNx Mean ± SEM N=10	0.61± 0.07^{a}	0.44± 0.05^{ac}	0.43± 0.04^{ac}	0.38 ± 0.03^{ac}	0.37± 0.03^{ac}
	STNx+M Mean ± SEM N=10	0.79± 0.06^{b}	0.60± 0.04^{bc}	0.56 ± 0.04^{bc}	0.50± 0.05^{c}	0.55 ± 0.04^{bc}

a: Significance by LSD at P <0.05 relative to sham control group.
b:Significance by LSD at P <0.05 relative to STNx group.
c: Significance at each record, 5, 15, 25&30 minutes of reperfusion relative to baseline values

Table (7): Percentage of change for the *in vitro* studied parameters at 5minute reperfusion record (early stage) and at 30 minute reperfusion record (late stage) compared to pre-ischemic basal record.(-ve sign=decreased % while +ve sign =increased %)

	5 min. perfusion			30 min perfusion		
	Sham Control	STNx	STNx +M	Sham Control	STNx	STNx +M
HR	-31.1± 4.3	-29.5 ± 4.6	-19.4± 4.9	-35.4 ± 5.86	- 44.5± 4.17	- 30.55± 4.22^{b}
PT	-28.9±6.3	-35.5 ± 3.7	-16.3 ± 3.3^{b}	-38.5± 4.95	-39.75± 5.98	-31.58 ± 5.17^{b}
PT/LVW	-31.8 ± 5.3	-35.6±3.8	-17.0 ± 3.8^{b}	-38.5 ±5.0	- 39.7 ± 6.1	-25.3 ± 4.4^{b}
TPT	+26.2± 10.9	+16.4 ±5.9	+20.4± 5.7	+36.5± 7.85	+51.93± 8.16	+20.28± 2.59^{b}
HRT	+22.04±6.1	+14.9±4.3	+12.0±2.7	+51.55± 14.0	+23.35± 5.63	+18.25±3.77
MFR	- 14.1 ±3.11	- 26.97±3.1^{a}	-23.5 ± 3.8	- 31.42± 4.06	- 35.62± 4.5	-36.39±4.97
MFR/ LVW	- 11.7 ±2.5	- 26.8 ± 3.4^{a}	- 23.5 ±3.7	- 29.5 ±4.2	-36.0± 4.2	- 29.6 ± 3.3

a: Significance by LSD at P <0.05 relative to sham control group.
b: Significance by LSD at P <0.05 relative to STNx group

On the other hand, melatonin treated group exhibited less deterioration of almost all of the hemodynamic parameters with reperfusion, in particular at 30min reperfusion.

Regarding the chronotropic activity, at 30 minute reperfusion, a 44.5± 4.17 % significant (P<0.05) decrease in HR in STNx versus 30.5 ± 4.2 % decrease in HR in melatonin treated has been observed.

Also, at 5 minute reperfusion, a 35.5 % significant (P<0.05) decrease in peak tension in STNx rats met with a 16 % decrease in tension in melatonin treated group. Moreover, in the late stage of reperfusion (at 30 min reperfusion) a 39.7 % significant (P<0.05) decrease in peak tension in STNx rats met by a 24.9% decrease in tension in melatonin treated group.

Melatonin treatment also exhibited significant (P<0.05) less decrease in peak tension / 100mg of LVW with reperfusion compared to non treated group. At 5 min reperfusion the decrease (P<0.05) in corrected peak tension was 35.6% in STNx rats versus 17.0% decrease in STNx + M group. At 30 min. reperfusion the decrease (P<0.05) in corrected peak tension was 39.7 % in STNx rats versus 25.3% decrease in STNx+ M rats.

The time to peak tension at 30min record showed a +51.9% prolongation in STNx rats compared to its basal records versus +20.3 % increases(P<0.05) in melatonin treated group compared to their basal record.

However in evaluating the % of change from the basal record in the STNx and STNx+M groups, in early and late stage of reperfusion the% of increase in HRT was nearly similar in both group (at 5 min :+14.9% in STNx group and + 12.0 % in STNx +M group, at 30 min.: +23.3% in STNx group versus + 18.25 % in STNx+M group) with non significant % of change.

Results of correlation:

As shown in table (8-a);correlation analysis in STNx group showed significant(P <0.03,P<0.01 &P<0.02) negative correlation between plasma MDA level in one side and plasma nitrate , cardiac tissue nitrate and GSH levels in other side respectively. Also, a positive significant (P<0.01) correlation between plasma MDA level and LV/RV ratio is observed together with positive correlation (though non significant) with LV/BW ratio. In addition, plasma nitrate showed a positive significant (P<0.02) correlation to cardiac tissue nitrate.

In addition, positive significant(r=0.645, P<0.04,n=10) correlation between the TPT and the whole heart /body weight ratio was observed in STNx group but not shown in the table.

As shown in table (8-b); correlation analysis in STNx+M group showed significant (P<0.009, P<0.001& P< 0.0001) negative correlation between plasma MDA and plasma & cardiac tissue nitrate as well as with cardiac tissue GSH respectively. Also, tissue nitrate level was positively (P<0.001) correlated with tissue GSH.

Table (8-a):Correlation analysis in nephrectomized group:

		urea	**Creatinine**	**Plasma nitrate**	**Tissue nitrate**	**Tissue GSH**	**LV/RV**	**LV/BW**
MDA	r	+0.057	+0.211	-- 0.674[a]	-- 0.757[a]	-- 0.707[a]	+0.764[a]	+0.270
	P	<0.87	<0.558	<0.033	<0.011	<0.022	<0.01	<0.45
	N	10	10	10	10	10	10	10
		urea	**Creatinine**	**Tissue Nitrate**	**Basal MFR**	**Flow/100gm LVW**		
Plasma Nitrate	R	-- 0.233	-- 0.041	+0.685[a]	+0.103	+0.062		
	P	<0.517	<0.911	<0.029	<0.776	<0.865		
	N	10	10	10	10	10		

Table (8-b): Correlation analysis in nephrectomized melatonin treated group.

		Urea	**Creatinine**	**Plasma nitrate**	**Tissue nitrate**	**Tissue GSH**	**LV/RV**
MDA	R	+0.133	+0.188	--0.769[b]	--0.869[b]	--0.973[b]	+0.689
	P	<0.714	<0.602	<0.009	<0.001	<0.0001	<0.27
	N	10	10	10	10	10	10
		urea	**Creatinine**	**Plasma nitrate**	**Tissue GSH**	**Basal MFR**	
Tissue Nitrate	R	- 0.009	-0.279	+0.614	+0.892[b]	+0.357	
	P	< 0.98	< 0.435	0.59	0.001	0.312	
	N	10	10	10	10	10	

a: Significance by LSD at P <0.05 relative to sham control group.

b: Significance by LSD at P <0.05 relative to STNx group

4-Discussion:

The ability of the heart to resist ischemic reperfusion injury either due to previous exposure to brief attacks of ischemia or due to any protocol applied before prolonged ischemia that protect the heart during ischemic reperfusion (Preconditioning), is very significant and important precaution other than cardioprotective drugs which are given during the cardiac attack, especially against acute episodes of myocardial infarction. As the onset of the attack is not predictable and cardioprotective intervention can usually implemented only at the time of reperfusion, after the appearance of the symptoms, where significant part of the damage has already occurred so preconditioning is of great therapeutic significance (Bolli 2007). Aforementioned findings, it is of interest to clarify the actual role of melatonin whether it has a cardioprotective effect or it abrogate the supposed preconditioning effects of free radicals generated during renal failure.

In this study, the increased urea and creatinin level in 5/6 partial nephrectomized rats confirming that partial nephrectomy had caused renal failure. On the other hand melatonin treatment significantly ameliorated the detrimental changes in both urea and creatinine. However, their values still higher compared to sham control, which indicate that the ability of melatonin to ameliorate the effect of nephrectomy is not complete. In coincide with this study the deterioration of renal function (plasma creatinine and proteinuria) and structure (glomerulosclerosis and tubulointerstitial damage) resulting from renal ablation was ameliorated significantly with melatonin treatment (Quiroz *et al.,* 2008).

The significantly, increased plasma level of MDA as an indicator for lipid peroxidation and free radical generation, and decreased plasma nitrate with partial nephrectomy could be correlated to each other. Reduction in nitric oxide levels during kidney failure has been related to the reaction of nitric oxide with superoxide anions to yield peroxynitrite rather than nitrate. Peroxynitrite possesses the biological activity responsible for renal damage (Kim et al., 2000 and Wang et al., 2003). This explanation is favored by the significant negative correlation between MDA values and levels of plasma nitrate demonstrated in 5/6 nephrectomized rats($r=0.674$ $p< 0.033$ $n=10$). In contradiction with our result, increased NO production in the systemic circulation of uremic rats (Aiello et al., 1997) and patients (Lau et al., 2000)was noticed.

Melatonin treatment significantly reduced MDA level which suggests an attenuated lipid peroxidation. Melatonin reported to be effectively protects against lipid peroxidation as well as decrease the synthesis of MDA which is an end product of lipid per oxidation (zwirski-korczala *et al.,* 2005).

Melatonin also significantly increased plasma nitrate concentration which implies slight improvement in NO level as nitrate level was still significantly low compared to sham control. The increased plasma nitrate as well as cardiac tissue nitrate with melatonin treatment could be attributed to the ability of melatonin to stimulate the endothelial NO synthesis through increasing intracellular calcium and/or increasing NO availability by preventing the NO conversion to peroxynitrite through its free radical scavenging activity (Pogan *et al.,* 2002). The reduced MDA together with an increase in NO levels after melatonin treatment as well as the significant negative correlation between them may be supportive for the previous explanation.

Regarding the basal cardiac properties in the form of significantly altered basal cardiac hemodynamic in nephrectomized non treated rats which is manifested in the significantly diminished basal peak tension and tension per LVW as well as in the significantly prolonged time to peak tension, all quantify myocardial systolic dysfunction with nephrectomy**.** Previous study reported an alteration in Ca2+ handling and an increase in the rate of inactivation of the L-type Ca2+current in isolated cardiac myocytes from STNx rats (Donohoe *et al*., 2000).

In addition, the significantly prolonged half relaxation time in nephrectomized non treated group may quantify diastolic impairment. Both systolic and diastolic dysfunctions have been reported before with renal failure which was attributed to the associated left ventricular hypertrophy (Zoccali *et al.,* 2004). In this study, the significantly increased left ventricular and whole heart weights to body weight ratios in nephrectomized non treated rats as well as the significantly reduced myocardial flow rate implies non physiological hypertrophy and may explain the systolic and diastolic impairment. Abnormalities of the microcirculation or of the main coronary circulation with uremia may lead to ischemia and fibrosis and to stiffness of the myocardium with subsequent occurrence of diastolic dysfunction (Malik *et al.,* 2009).

The significantly prolonged time to peak tension and half relaxation time in nephrectomized rats is reminiscent with the simultaneous negative chronotropic effect, since these times represent parts of the prolonged duration of the whole cycle. Moreover, the positive significant correlation between the TPT and the whole heart /body weight ratio in STNx group may explain this prolonged time to reach peak tension($r=0.645$, $p< 0.04$, $N=10$)

The significantly reduced basal myocardial flow rate in nephrectomized rats could be attributed to the increased liability of ischemic heart diseases due to reduction in coronary reserve and capillary density with chronic kidney diseases (Amann *et al.,* 1998).Also the highly significant($p<0.0001$ $r=0.614$) positive correlation between the coronary flow and the plasma nitrate level in all groups and positive though non significant in STNx group all could lead to a suggest that reduced plasma nitrate level and hence its reduced vasodilator effect in nephrectomized rate may be compromised in this reduced basal coronary flow.

In contradiction with our result, in acute experimental uremia several indices of myocardial contractility have been found to be increased but the experimental model was acute rather than chronic (Nivatpumin *et al.,* 1975). Also, some studies showed structural changes but no cardiac functional changes with uremia (Hatori *et al.,* 2000; kennedy *et al.,* 2006). However, in line with our result , cardiac function in an isolated perfused working heart ,was significantly impaired 4 weeks after 5/6 nephrectomy (raine *et al.,* 1993) .

The baseline activity of hearts isolated from nephrectomized melatonin treated rats(STNx+M) was not significantly different from that of nephrectomized non treated rats as regards heart rate, peak tension development, time to peak tension as well as half relaxation time.

Melatonin treatment improved the basal coronary flow rate significantly compared to the STNx rats but it caused border line significant increase in MFR/100 mg LVW which could be attributed to the inability of melatonin to ameliorate completely the cardiac hypertrophy associated with renal failure. Thus melatonin treatment concomitant with renal impairment did not alter the basal intrinsic properties of the heart, but its effect on the baseline coronary flow may be due to its vascular effect.

Melatonin showed to inhibit the development of nitrate tolerance (diminished blood vessel responsiveness to the vasodilator effect of nitroglycerin) in coronary arteries via MT1- or MT2-melatonin receptors and this requires the presence of endothelial cells (O'Rourke *et al.,* 2003)or via increasing NO availability which stimulates guanylate cyclase in smooth muscle cells leading to vasodilatation (Pogan *et al.,* 2002).

In all studied groups the pattern of response to reperfusion after a period of ischemia was in the form of decreasing in beating rate (HR), depression in peak tension (PT), diminished myocardial flow rate (MFR) as well as significantly prolonged time to peak tension (TPT) and half relaxation time (HRT). This response varied in intensity in the three studied groups. Also, it varied during early (5 min.) and late stage (30 min.) of reperfusion. during ischemia ATP declines resulting in decrease in PH due to anaerobic glycolysis. The increased proton stimulates Na+- H+ exchanger resulting in increased intracellular Na+ which reverse the mode of Na+- Ca ++ exchanger leading to increased intracellular and intra mitochondrial Ca ++ level. This in turn activates channel protein (MPT) in inner mitochondrial membrane, leading to loss of ATP which falls to very low levels that causes inhibition of some process and cellular death In addition, generation of reactive oxygen species (ROS) during ischemia may play a role in this impairment. However the generation of ROS during ischemia is very low as oxygen is required for this process (Murphy and Steenbergen 2008)..

On the other hand during reperfusion, a large burst of reactive oxygen species (ROS) has been consistently shown to occur with extensive tissue damage. Cardiac myocytes, endothelial cells, and infiltrating neutrophils contribute to this enhanced ROS production. These radicals may exceed the capacity of the cellular intrinsic free radical scavenging systems (Petrosillo *et al.,* 2006 and Murphy & steenbergen 2008). The altered cardiac hemodynamic with ischemic reperfusion agree with other findings where depressed contractile function, coronary flow as well as altered vascular reactivity occurs with I/R (Zweier *et al.,* 2006).

In this study , 5/6 nephrectomized rats showed more liability for reperfusion injury which may be related to the observed reduced cardiac GSH(anti-oxidant)which may be exhausted in defending the free radicals supposed to associate renal damage and could be dictated by the increased plasma MDA level. Also the reduced plasma and cardiac tissue nitrate levels may quantify NO involvement in peroxynitrite formation. The negative significant correlation between MDA level and cardiac tissue GSH & both plasma and cardiac tissue nitrate level is supportive to the previous explanation.

Despite of the previously mentioned deteriorated cardiac activity in STNx rats during reperfusion of their hearts, the % of change in all of its cardiac parameters from the basal record at 5 min. and 30 min not differ significantly from that percentage of change in sham control hearts (table 7). This may refers to possibility of preconditioning but the increased free radicals dictated in increased MDA level in STNx group could not provide the suspected preconditioning effect suggested by other studies (Yellon and Downey 2003; Genade *et al.,* 2006) which appeared markedly in the significantly reduced myocardial flow rate and significant decrease in myocardial flow rate/ left ventricular weight. Thus the preconditioning effect of free radical could be suggested to be inconclusive.

Melatonin treatment improved the deteriorated chronotropic activity in STNx+ M group both in early stage of reperfusion (though non significant) and late stage of reperfusion (significant). Also it improved the inotropic activity manifested in the significantly better % of change in PT& PT/100mg LVW both at early and late stage of reperfusion. Also this was manifested in shorter time to peak tension with melatonin treatment. Thus melatonin could be suggested to provide preconditioning and /or protection against ischemic reperfusion injury.

Preconditioning was reported to occur in two phases, very early phase within a few minutes after the preconditioning stimulus and lasts only 1–2 hours. The second window of protection develops more slowly, 12–24 hours after the preconditioning stimulus, but it lasts much longer, for 3–4 days and requires the synthesis of new proteins (Liem et al., 2007). The effect of melatonin could be suggested in this study to stimulate the late phase preconditioning, and increasing cardiac tissue GSH and nitrate may be their possible tools.

On the other hand, melatonin did not show significant better % of change in half relaxation time (HRT) or coronary flow rate during reperfusion which suggests its inability to abrogate the diastolic dysfunction and the impaired myocardial flow rate which observed in nephrectomized non treated rats during reperfusion. In other study, it was reported that reversibly injured regions in heart can demonstrate persistent diastolic dysfunction despite complete systolic functional recovery 24 h after reperfusion, demonstrating a direct relationship between micro vascular obstruction and greater post-infarct LV diastolic dysfunction (Azevedo *et al.*, 2004).

In addition to the effect of melatonin to decrease the liability for reperfusion injury in this study , melatonin also in other studies reduced myocardial I/R injury (Reiter and Tan 2003 and Ceyran *et al.*, 2008) and decreased the myocardial infarct size in isolated rat heart (Sahna *et al.*, 2005)

Cardiac tissue reduced glutathione (GSH), as anti oxidative defense system, was reduced in I/R hearts in nephrectomized rats, but it was significantly increased in the nephrectomized melatonin-treated group. In line with this result, Melatonin was reported not only to supports several intracellular enzymatic antioxidant enzymes, including SOD and glutathione peroxidase (GSH-Px) but also induces the activity of γ-glutamylcysteine synthetase, thereby stimulating the production of another intracellular antioxidant glutathione (GSH). (Winiarska *et al.*, 2006)

The significantly decreased cardiac tissue nitrate in nephrectomized rats reflects the effect of I/R. ischemia-reperfusion can induce endothelial cell injury and activate endogenous NOS inhibitors, leading to eNOS activity inhibition and NO production dysfunction. Reperfusion may produce also significant reactive oxygen species, such as the superoxide anion and hydroxyl radicals, which damage lipids and proteins. NO is a strong antioxidant and can substantially be degraded by reacting directly with free radicals. (Sun, *et al.*, 2009)

On the other hand, the significant increase in cardiac tissue nitrate, an indicator for nitric oxide level, with melatonin treatment may be considered as a triggering factor in preconditioning. NO has been shown to have an important role in PC and cardioprotection (Bolli 2001; Jones and Bolli 2006 and Murphy & Steenbergen 2008) plausible mechanisms whereby NO enhances resistance to cell death following ischemia-reperfusion include inhibition of calcium influx, antagonism of adrenergic stimulation, reduction in myocardial oxygen demands, opening of sarcolemmal and/or mitochondrial ATP-sensitive K(KATP) channels, and possibly direct antioxidant actions, such as inhibition of the effects of superoxide anion (O2•) and peroxynitrite (ONOO) (Bolli , 2007). Pretreatment with melatonin increased NO bioavailability and decreased endothelin expression and consequently suggested to play a protective role in preserving both liver function and structure during ischemia and reperfusion injury (Zhang *et al.*, 2006) Moreover, it was postulated that the fundamental role of NO is in late PC: that is, NO plays a dual role in the late phase of this phenomenon, acting initially as the trigger and subsequently as the mediator of late PC (Bolli 2007).

The whole heart and left ventricular hypertrophic changes in the nephrectomized group is in line with the results in multiple studies. Cardiovascular remodeling has been described in STNx rats (Törnig *et al.*, 1996) and in uremic patients (Amann *et al.*, 1998). Also, cardiac hypertrophy and histological evidence of myocardial fibrosis has been seen in absence of hypertension 8 weeks after subtotal nephrectomy in rats (Donohoe *et al.*, 2000). Cardiac hypertrophy may be due to the associated volume overload leading to eccentric hypertrophy with a resultant myocyte to arteriolar capillary mismatch or due to the possible associated hypertension or higher angiotension II concentration leading to concentric hypertrophy (Berl and Henrich 2006) .

The ability of melatonin treatment to ameliorate these hypertrophic changes may be attributed to its anti- oxidant capacity. A previous study showed that oxidative stress plays an important role in cardiac hypertrophy (Araujo *et al.*, 2008).

Also the increased cardiac tissue nitrate, an indicator for nitric oxide level in melatonin treated group may be contributed in the reduced cardiac hypertrophy. NO has been shown to inhibit human

endothelial cell apoptosis and Myocardial apoptosis reported to be one of many important factors leading to cardiac remodeling as apoptosis is followed by compensatory hypertrophy (Sata *et al.*, 2000 and Agata *et al.*, 2002) however the effect was not sufficient compared to normal control rats.
Melatonin was found to prevent cardiac hypertrophy in hyperthyroid rats which may occurs as a result of the effect of melatonin on hemodynamic overload, NO availability, free radicals and lipid profile which were suggested all to modify myocardial remodeling (Ghosh *et a.*, 2007)

5-Conclusion:

The increased MDA which is an indicator for free radical generation in partial nephrectomized rats did not provide the supposed preconditioning effect against ischemic reperfusion injury in isolated hearts later on or its effect was inconclusive. Also, melatonin as a cardio-protective therapy improved the impairment of coronary flow rate due to renal failure under basal condition, but it was unable to ameliorate the basal systolic and diastolic dysfunction. However with I/R a situation of generation of more free radical, melatonin treatment exhibited less deterioration in the chronotropic and inotropic activity compared to nephrectomized non treated group. Melatonin did not support the diastolic dysfunction compared to its effect on systolic function. In addition, long term therapy of melatonin concomitant with deterioration of kidney function, appears to offer some sort of preconditioning and protection against I/R injury in particular during late reperfusion. Cardiac tissue GSH (anti-oxidant) and NO triggering by melatonin may be added to its free radical scavenging effect in the suggested protection and / or preconditioning.

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Evaluation of Serum Chromogranin A as a Useful Tumor Marker for Diagnosis of Hepatocellular Carcinoma

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Abstract: Background: In Egypt, HCC was reported to account for about 4.7% of chronic liver disease patients. Approximately 80% of HCCs are associated with cirrhosis, which is regarded as the most important precancerous etiological factor. Chromogranin A is a cellular marker for neuroendocrine tumors. High serum levels of CgA have also been demonstrated in patients with other malignancies including colon, lung, breast and prostate cancer. Objective: To evaluate serum CgA as a marker for HCC. Patients and Methods: Eighty cases (30 with HCC, 30 with liver cirrhosis and 20 apparently healthy controls) were subjected for estimation of Chromogranin A (CgA) and Alpha feto protein (AFP) by ELISA technique together with routine laboratory investigations including CBC, prothrombin time and concentration and INR and serum urea, creatinine, albumin, AST, ALT, alkaline phosphatase and bilirubin (total and direct). Results: There was a highly significant statistical difference between control group and HCC group and between liver cirrhosis group and HCC group as regard to AFP and Chromogrnin A ($P<0.01$). There was a significant statistical difference between control group and liver cirrhosis group as regard to AFP and Chromogrnin A ($P<0.05$). Conclusion: the results of the present study revealed that the application of CgA as a tumor marker in the diagnosis of HCC is to be considered especially in cases with low levels of AFP, as determination of CgA serum values represents a complementary diagnostic tool in monitoring chronic liver disease patients for detection of HCC. The combined use of both CgA and AFP to detect HCC increases their sensitivity and specificity.

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Keywords: Chromogranin A, Hepatocellular carcinoma, liver cirrhosis.

1. Introduction:

Hepatocellular carcinoma (HCC) is the most common cause of primary liver neoplasms and the fourth most frequent type of cancer worldwide following lung, breast and bowel cancers with an increasing incidence, causing one million deaths per year[(1)].

A study conducted at Cairo Liver Center, a specialized center for the study and management of liver diseases, revealed that HCC has nearly doubled over the last decade and there is a growing incidence of HCC in Egypt (10–120 cases/ 100,000), which represents the leading cause of death from all other cancer sites[(2)].

In Egypt, HCC was reported to account for about 4.7% of chronic liver disease (CLD) patients[(3)] but there is a remarkable increase in the proportion of HCC among CLD patients from 4.0% to 7.2% over the last decade[(4)]. This rising proportion may be explained by the increasing risk factors such as the emergence of HCV over the same period of time, the contribution of HBV infection, improvement of the screening programs and diagnostic tools of HCC[(5)]. Age distribution among HCC patients revealed that the most predominant age is (40-59) years[(4)].

Approximately 80% of HCCs are associated with cirrhosis, which is regarded as the most important precancerous etiological factor. Hepatocyte necrosis and subsequent increased proliferation due to chronic hepatitis favor nodular regeneration, which might be followed by hepatocyte dysplasia and possibly HCC[(6)]. The causes for hepatocyte necrosis may be infectious, toxic, metabolic, and autoimmune. The majority of HCV-infected individuals become chronic carriers of the virus, and long term follow-up studies have demonstrated that a proportion (4%-25%) develops cirrhosis and ultimately HCC. Due to the silent progression of cirrhosis towards HCC, there is a clear need for a marker capable of detecting the transformation[(7,8)].

Alpha fetoprotein (AFP) is the most established tumor marker in HCC and the gold standard by which other markers for the disease are judged[(9)].

AFP is a fetal specific glycoprotein synthesized from fetal yolk sac, liver and intestines. Normally, its serum concentration falls rapidly after birth and its synthesis in adult life is depressed. However, more than 70% of HCC patients have high serum concentrations of AFP because of tumor excretion. Forty years after its discovery, serum AFP

remains the most useful marker for screening HCC patients. The normal range for serum AFP levels is up to 20 ng/ml [10].

Mild elevations of AFP can be seen in benign liver diseases such as virus related acute and chronic hepatitis[11].

Among patients with chronic hepatitis C, serum AFP values are frequently elevated, even in the absence of HCC. Factors associated with raised AFP include severity of liver diseases, female gender and black race[12].

Chromogranin A (CgA) is an acidic, hydrophilic protein of 439 amino acids (49 kDa), present in chromaffin granules of the neuroendocrine cells. CgA acts as a pro-hormone and its proteolysis constitutes a key element of its physiology. This degradation releases biologically active peptides (vasostatins, chromostatin, pancreastatin, paraststin, etc.) that have different paracrine and autocrine functions. The proteolysis is tissue specific, and the protein's fragmentation differs depending on its location. Although the function of CgA is not well known, it seems to be related to calcium binding activity[13].

Low levels of CgA in the circulation are present in healthy subjects and are independent of age and sex. The importance of increased CgA levels in serum was first shown in patients with pheochromocytoma, and then demonstrated in other endocrine cancers[14]. High serum levels of CgA have also been demonstrated in patients with other malignancies including colon, lung, breast and prostate cancer, possibly in relation to a neuroendocrine differentiation[15]. Interestingly, clusters of cells containing CgA have been demonstrated within HCC tissue[16] and recent studies reported elevated levels of serum CgA in HCC patients, suggesting a possible diagnostic role of this marker[17].

The present study was aimed at comparing serum CgA concentration in HCC patients and those with cirrhotic liver disease to assess the potential usefulness of this marker in diagnosis of HCC.

2. Subjects and Methods:

This study was conducted on 60 subjects admitted to Hepatology, Gastroenterology and Infectious Diseases Department, Benha University Hospital, during the period from April 2009 to April 2010. They were classified into 2 groups. The first group included 30 patients with hepatocellular carcinoma (HCC) on top of liver cirrhosis, 16/30 (53%) of them were males and 14/30 (46.7%) were females, their ages ranged from 42-70 years (mean of 54.27 ± 6.4 years). The second group included 30 patients with liver cirrhosis on top of chronic liver disease, 18/30 (60%) of them were males and 12/30 (40%) were females, their ages ranged from 40-70 years (mean of 54.2 ± 9 years). A third group of 20 apparently healthy subjects serving as control group were also included in this study, 10/20 (50%) of them were males and 10/20 (50%) of them were females, their ages ranged from 42-66 years (mean of 54.10± 6.9 years), they were clinically free with normal laboratory findings and negative viral hepatitis markers and normal abdominal ultrasonogrphic findings. Informed written consent was obtained from all participants. Patients with heart failure, kidney failure and carcinoma elsewhere were not included, since these conditions may be associated with increased levels of CgA.

The diagnosis of HCC cases was done by:

1. Focal lesion in the liver in abdominal sonography.
2. Enhancement of focal lesion on abdominal triphasic C.T.
3. Typical hsitopathological findings. The lesions were of grade I histopathologically in 2 patients (6.7%), of grade II in 25 patients (83.3%) and of grade III in 3 patients (10%).

The diagnosis of HCV infection was defined by positive tests for antibodies against HCV, based on an enzyme immunoassay. The diagnosis was confirmed by the presence of detectable HCV RNA in the circulation by polymerase chain reaction (PCR). Diagnosis of HBV was determined by HBsAg commercial enzyme immunoassay kits and confirmed by measurement of HBV DNA in serum by PCR. Diagnosis of Bilharziasis was done by IHA and confirmed by rectal snip.

All studied individuals were subjected to the following:

I. Full history and clinical examination.

II. Laboratory investigations:

(A) Routine investigation.

1. CBC using automated blood counter (Sysmex KX. 21 N).
2. Prothrombin time and concentration and INR using (Option 4 coagulometer) and (DiaMed "Dia Plastin" reagent)
3. Serum chemistry by (Bs-300 automated chemistry analyzer) including: urea, creatinine, albumin, AST, ALT, alkaline phosphatase and bilirubin (total and direct)

(B) Tumor markers:

1. Chromogranin (A).
2. Alpha feto protein.

Samples:

- Blood samples were obtained by peripheral venipuncture from patients.
- One sample was taken.

Table (1): Characteristics of liver cirrhosis and HCC groups according to viral hepatitis markers and antibilharzial antibody:

Parameter		Liver cirrhosis		HCC		Total
		NO.	%	NO.	%	NO.
Hepatitis C	+ve	22	73	27	90	49
	-ve	8	27	3	10	11
Hepatitis B	+ve	11	37	7	23	18
	-ve	19	63	23	77	42
Bilharziasis	+ve	6	20	5	17	11
	-ve	24	80	25	83	49
Hepatitis C & B	+ve	3	10	4	13	7
Hepatitis C & Bilharziasis	+ve	2	6	5	17	7
Hepatitis B & Bilharziasis	+ve	4	13	0	0	4

- The blood sample obtained was divided as follow:
  1. 1 ml of blood on 15 µL EDTA to perform CBC.
  2. 2.25 ml of blood on 250 µL sodium citrate to perform prothrombin time.
  3. 5 ml of blood was taken in plain tube then put in water bath at 37 ^{o}C for 30 minutes then centrifuged for 10 minutes then the resultant serum was divided into two aliquots. The first aliquot was used for routine investigations. The second aliquot was kept frozen at -20 C° for measurements of chromogranin A and alph-feto protein.

Serum CgA was assayed by a commercial kit from DRG International Inc., USA. The assay utilizes the two-site "sandwich" ELISA technique with two selected antibodies that bind to different epitopes of human chromogranin A.

Assay standards, controls and patient samples were directly added to microtiter wells of microplate that was coated with a polyclonal chromogranin A antibody. After the first incubation period, the antibody on the wall of microtiter well captured human chromogranin A in the sample and unbound antibodies in each microtiter well was washed away. Then a horseradish peroxidase (HRP) labeled monoclonal anti-human chromogranin A antibody was added to each microtiter well and a "sandwich" of "monoclonal antibody - human chromogranin A – polyclonal antibody" was formed. The unbound monoclonal antibody was removed in the subsequent washing step. For the detection of this immunocomplex, the well was then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader at 450 nm.

The enzymatic activity of the immunocomplex bound to the chromogranin A on the wall of the microtiter well was directly proportional to the amount of chromogranin A in the sample.

A standard curve was generated by plotting the absorbance versus the respective human chromogranin A concentration for each standard on point-to-point curve fit. The concentration of human chromogranin A in test samples was determined directly from this standard curve.

Serum AFP was measured by enzyme immunoassay (EIA) using human AFP EIA kit provided by DIMA Company, Germany. The test is based on simultaneous binding of human AFP to two monoclonal antibodies, one immobilized on microwell plates, the other conjugated with horseradish peroxidase. After incubation, the bound/free separation was performed by a simple solid-phase washing, and then the substrate solution (TMB) was added. After an appropriate time was elapsed for maximum color development, the enzyme reaction was stopped and the absorbance was determined at 450 nm against blank. The AFP concentration in the sample was calculated based on a series of standards. The color intensity was proportional to the AFP concentration in the sample.

Statistical Methods:

The collected data was organized, tabulated and statistically analyzed using SPSS software statistical computer package version 16. For quantitative data, mean and standard deviation were

calculated. Student "t" test: used to test the significance of the difference between two groups. Qualitative data was expressed as frequency and percentage.

Chi square test: to compare between qualitative parameters.

The association of serum chromogranin A level with continuous variables was tested with Pearson's correlation. P value was considered significant if < 0.05 & not significant if > 0.05.

3. Results

The results of the present study are summarized, statistically analyzed and presented in the following tables and figures.

Table (2): Blood picture and INR in control, liver cirrhosis and HCC groups:

Parameter	Group	N	Median	t		p	
Hb (gm/dl)	control	20	12.6	t_1	5.9	P_1	<0.01
	Liver cirrhosis	30	10.5	t_2	4.9	P_2	<0.01
	HCC	30	11	t_3	1.7	p_3	>0.05
WBCs (x 10^9 /L)	control	20	7.1	t_1	5.3	P_1	<0.01
	Liver cirrhosis	30	4.0	t_2	4.7	P_2	<0.01
	HCC	30	4.1	t_3	0.3	p_3	>0.05
Platelets (x 10^9 /L)	control	20	104.5	t_1	4.03	P_1	<0.01
	Liver cirrhosis	30	172	t_2	7.4	P_2	<0.01
	HCC	30	108	t_3	5.1	p_3	<0.01
INR	control	20	1	t_1	4.6	P_1	<0.01
	Liver cirrhosis	30	1.5	t_3	6.5	P_2	<0.01
	HCC	30	1.6	t_3	2.03	p_3	>0.05

t_1 & p_1 between control and liver cirrhosis.
t_2 & p_2 between control and HCC.
t_3 & p_3 between liver cirrhosis and HCC.
p value > 0.05 is considered non significant.
p value < 0.05 is considered significant.
p value < 0.01 is considered highly significant.

There was a highly significant statistical difference between control group and liver cirrhosis group and between control group and HCC group as regard Hb concentration, WBCs count, platelet count and INR (P<0.01) (table 2).

There was a highly significant statistical difference between HCC group and liver cirrhosis group as regard to platelet count (P<0.01) (table 2).

There was non significant statistical difference between HCC group and liver cirrhosis group as regard Hb, WBCs count and INR (p>0.05) (table 2).

There was a highly significant statistical difference between control group and liver cirrhosis group as regard AST, ALT, alkaline phosphatase, total bilirubin, direct bilirubin and albumin (P<0.01) (table 3).

There was a highly significant statistical difference between control group and HCC group as regard AST, ALT, alkaline phosphatase and albumin (P<0.01) (table 3).

There was a significant statistical difference between control group and HCC group as regard total bilirubin and direct bilirubin (P<0.05) (table 3).

There was a highly significant statistical difference between HCC group and liver cirrhosis group as regard AST (P<0.01) (table 3).

There was a significant statistical difference between HCC group and liver cirrhosis group as regard ALT, alkaline phosphatase and albumin (P<0.05) (table 3).

There was non significant statistical difference between HCC group and liver cirrhosis group as regard total bilirubin and direct bilirubin (P>0.05) (table 3).

There was a significant statistical difference between control group and liver cirrhosis group as regard AFP and Chromogrnin A (P<0.05) (table 4).

There was a highly significant statistical difference between control group and HCC group and between liver cirrhosis group and HCC group as regard AFP and Chromogrnin A (P<0.01) (table 4).

Table (3): Levels of to AST, ALT, and Alk.phosphatase, albumin, total bilirubin and direct bilirubin in control, liver cirrhosis and HCC groups.

Parameter	Group	N	Median	t		p	
AST (U/L)	control	20	27.5	t_1	15.5	p_1	<0.01
	Liver cirrhosis	30	67.5	t_2	13.6	p_2	<0.01
	HCC	30	95	t_3	6.2	p_3	<0.01
ALT (U/L)	control	20	24	t_1	12.9	p_1	<0.01
	Liver cirrhosis	30	56	t_2	9.3	p_2	<0.01
	HCC	30	58	t_3	2.9	p_3	<0.05
Alkaline phosphatase (U/L)	control	20	90	t_1	2.9	p_1	<0.01
	Liver cirrhosis	30	114.5	t_2	3.3	p_2	<0.01
	HCC	30	122	t_3	2.6	p_3	<0.05
Albumin (g/dl)	control	20	4.25	t_1	3.9	p_1	<0.01
	Liver cirrhosis	30	3.6	t_2	4.8	p_2	<0.01
	HCC	30	3.4	t_3	3.5	p_3	<0.05
Total bilirubin (mg/dl)	control	20	0.9	t_1	6.4	p_1	<0.01
	Liver cirrhosis	30	1.5	t_2	3.6	p_2	<0.05
	HCC	30	1.05	t_3	1.8	p_3	>0.05
Direct bilirubin (mg/dl)	control	20	0.2	t_1	5.1	p_1	<0.01
	Liver cirrhosis	30	0.4	t_2	3.4	p_2	<0.05
	HCC	30	0.3	t_3	0.2	p_3	>0.05

Table (4): Comparison between control, liver cirrhosis and HCC groups according to AFP & Chromogranin A:

Parameter	Group	N	Median	t		p	
AFP (ng/ml)	control	20	2.75	t_1	2.8	p_1	<0.05
	Liver cirrhosis	30	5.35	t_2	3.6	p_2	<0.01
	HCC	30	26.5	t_3	4.04	p_3	<0.01
Chromogranin A (ng/ml)	control	20	15.8	t_1	2.1	p_1	<0.05
	Liver cirrhosis	30	19.5	t_2	5.1	p_2	<0.01
	HCC	30	71.7	t_3	5.2	p_3	<0.01

Table (5): Sensitivity, specificity, PPV and NPV of AFP, Chromogranin A and both:

Parameter	sensitivity	specificity	PPV	NPV
AFP	86.7%	80%	81.3%	85.7%
Chromogranin(A)	83.3%	76.7%	78.1%	82.1%
AFP and Chromogranin(A)	90%	83.3%	81.8%	89.3%

PPV= Positive predictive value. NPV= Negative predictive value.

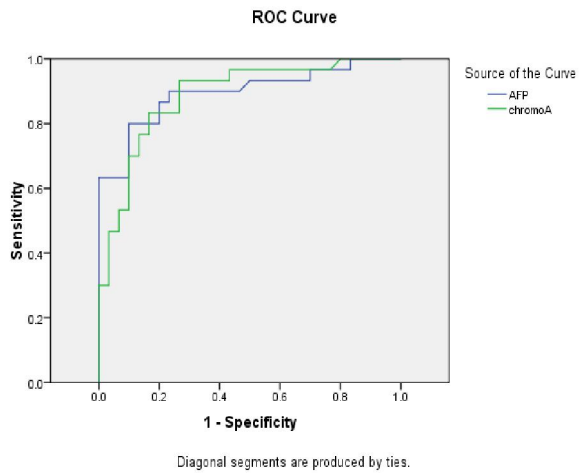

Figure 1: Comparison between AFP and Chromogrnin A by ROC curve.

Table (6): Area under the Curve for AFP and Chromogranin A

Test Result Variable(s)	AUC
AFP	0.895
Chromogranin A	0.886

* The more the area under the curve, the better is the test

Table (7): Correlation between AFP and different studied variables

Parameter	r	p
Chromogrnin A	-0.1	>0.05
Hb	-0.03	>0.05
WBCs	-0.03	>0.05
Platelets	0.2	>0.05
INR	0.5	<0.05
Creatinine	0.1	>0.05
Urea	0.4	>0.05
AST	0.1	>0.05
ALT	-0.02	>0.05
Alk.phosphatase	-0.1	>0.05
Total bilirubin	0.1	>0.05
Direct bilirubin	0.03	>0.05
Albumin	-0.1	>0.05

There was non significant correlation between AFP and Chromogranin A, Hb, WBCs, platelets, ALT, alkaline phosphatase, albumin, AST, total bilirubin and direct bilirubin, creatinine and urea (table 7).

There was a positive significant correlation between AFP and INR (table 7).

Table (8): Correlation between Chromogrnin A and different studied variables

Parameter	r	p
AFP	-0.1	>0.05
Hb	0.23	>0.05
WBCs	0.3	>0.05
Platelets	-0.04	>0.05
INR	0.14	>0.05
Creatinine	-0.06	>0.05
Urea	0.01	>0.05
AST	0.1	>0.05
ALT	-0.1	>0.05
Alk.phosphatase	0.85	<0.05
Total bilirubin	-0.1	>0.05
Direct bilirubin	0.3	>0.05
Albumin	-0.86	<0.05

There was non significant correlation between Chromogranin A and AFP, Hb, WBCs, platelets, INR, ALT, AST, total bilirubin and direct bilirubin, creatinine and urea (table 8).

There was a negative significant correlation between Chromogranin A and albumin (table 8).

There was a positive significant correlation between chromogranin A and alkaline phosphatase (table 8).

4. Discussion:

In Egypt, HCC is the third most frequent cancer in men with > 8000 new cases predicted by 2012[18]. Early detection of HCC opens doors for various effective treatments such as surgical resection, radiofrequency ablation, and transplantation, which can subsequently lead to long-term survivals in a great number of HCC patients[19].

In our study HCV as a cause of cirrhosis accounted for 90% of HCC patients reflecting the close relationship between HCV and HCC as one of prominent risk factors of developing HCC and this is in agreement with Montalto et al. [20] who reported that Liver cancer has a higher prevalence in patients with HCV-associated cirrhosis than in non-viral etiologies of chronic liver disease, while only a few cases of HCV-associated HCC have been reported in the non-cirrhotic liver, indicating that the virus possibly has a mutagenic effect.

In our study HBV carriers were 23% in HCC group and this is in agreement with Liu and Kao [21] who reported that HCC has been the first human cancer amenable to prevention using mass vaccination programs from global perspective. The burden of chronic HBV infection is expected to decline because of increasing utilization of HBV immunization since the early 1980 and this also in

agreement with El-Zayadi et al. [3] who stated that the relative risk of developing HCC for HBV carriers may be 100-200 fold higher than that for non-carriers however, the prevelance of HBV infection in Egypt has been declining over the last two decades.

Concerning hematological tests in this study, most of the patients with cirrhosis and HCC have significantly lower hemoglobin value and platelet count ($p < 0.01$) in comparison to the apparently healthy control group and this was in agreement with Franca et al. [22] who reported that there are various theories about thrombocytopenia in chronic liver diseases, portal hypertension, hypersplenism and bone marrow suppression are factors associated with thrombocytopenia.

On analysis of liver biochemical profile, there was significant deterioration in cirrhosis and HCC patients when compared to the control group and this was in agreement with Sleisenger and Fordtran [23] who reported that these tests will usually indicate the type of liver injury, whether hepatocellular or cholestatic, but cannot be expected to differentiate one form of hepatitis from another or to determine whether cholestasis is intra or extrahepatic.

In our study most of the HCC lesions on ultrasound (60%) were found in the right lobe of the liver and this was in agreement with that reported by Nihal et al. [24].

As regard the CT pattern of the HCC lesions in triphasic CT scan 26 lesions (86.7%) showed typical enhancement features of HCC (typical specific pattern of arterial uptake followed by venous washout in the delayed venous phase) this was in agreement with Peterson et al. [25] who performed a screening test on large populations of HCC patients before transplantation revealing that CT scanning showed typical enhancement pattern in 68% of HCC patients.

In this study histopathological assessment was done for patients with HCC revealing that 6.7% of patients were grade I, 10 % grade III and 83.3% were grade II and this was in agreement with that reported by Darwish [26] who noticed that grade II was more detected than the other two grades in biopsied HCC lesions.

Current diagnosis of HCC relies on clinical information, liver imaging and measurement of serum alpha-fetoprotein [27, 28].

In this study, there was statistically highly significant elevation ($p < 0.01$) in the median serum AFP in HCC group (26.5 ng/ml) when compared with control group (2.75 ng/ml) and highly significant elevation ($p<0.01$) when compared with cirrhotic group (5.35 ng/ml) and this is not in agreement with Massironi et al. [17], who reported no significant difference in AFP between HCC and liver cirrhosis and healthy subjects. In our study considering cut off value of 7.295 ng/ml (mean ± 2SD), the sensitivity of AFP was (86.7%) and the specificity was (80%). These results are in agreement with Massironi et al. [17] who reported a sensitivity of (75%) and specificity of (80%).

Our results revealed that there was a statistically highly significant elevation ($p<0.01$) in the median serum CgA in HCC group (71.7 ng/ml) when compared with control group (15.8 ng/ml) and highly significant elevation ($p <0.01$) when compared with cirrhotic group (19.5 ng/ml).

These results are in agreement with Leone et al. [17], Spadaro et al. [29] and Massironi et al. [17] who reported statistically significant elevation of CgA serum levels in HCC when compared to those in cirrhotic patients. Although it is not clear why a non neuroendocrinal tumor such as HCC express CgA, Bosman [30] noted that neuroendocrine differentiation can occur in carcinomas that lack neuroendocrine cells in their normal epithelial counterparts, such as hepatocellular carcinoma.

In our study considering the cut off value of 28.78 ng/ml the sensitivity of CgA was (83.3%) and the specificity was (76.7%). These results are in agreement with Massironi et al. [17] who reported a sensitivity of (70%) and specificity of (67%). The area under the rock curve for CgA was 0.886 compared to 0.895 for AFP.

In our results the combined use of the two markers AFP and CgA led to increase in the specificity of AFP and CgA from (80%) and (76.7%) respectively to (83.3%) and increase in the sensitivity of AFP and CgA from (86.7%) and (83.3%) respectively to (90%). These results were in agreement with Spadaro et al. [29] who concluded that when AFP is normal or < 200 ng/ml and in the presence of suspicious clinical, laboratory and or imaging signs of HCC, the evaluation of CgA levels becomes of particular importance in the follow up of chronic liver disease patients. This showed that simultaneous measurements of serum AFP and CgA are of value in detecting HCC.

Concerning the correlation between the levels of AFP and CgA there was no correlation between serum CgA and AFP in patients of HCC group. This was in agreement with Spadaro et al. [29] who reported no correlation between both markers in patients with HCC.

In conclusion, the results of the present study in keeping with evidences from literature revealed that the application of CgA as a tumor marker in the diagnosis of HCC is to be considered especially in cases with low levels of AFP, as determination of CgA serum values represents a

complementary diagnostic tool in monitoring chronic liver disease patients for detection of HCC. CgA could be combined with AFP to detect HCC, as the combined use of both markers increases their sensitivity and specificity.

A screening program for HCC detection is recommended to all patients with cirrhotic liver by tumor markers such as combined use of alpha-fetoprotein and chromogranin A and ultrasonography. However triphasic C.T and/or liver biopsy may be needed especially if the previous methods were not conclusive. On basis of these findings we are recommending further studies including a large number of patients to ascertain whether circulating CgA is useful as a prognostic marker and to evaluate its significance in the diagnosis of HCC. A follow-up of CgA serum values after treatment of HCC is also recommended in order to define the utility of the marker for the detection of recurrent tumor.

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Monte Carlo method and the Ising model for magnetized and non-magnetized water as MRI contrast agent

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Abstract: A Monte Carlo algorithm for a two dimensional Ising model is proposed and implemented using Mat lab. It describes a lattice with a discrete number of particles. We study the evolution of the system over time depending on a particular variable called the interaction strength .The results of computer simulations agree with practical experiments showing that there is a change in Energy-Magnetization and strength interaction-Magnetization curves between magnetized water and normal water which means that the magnetized water or Saline changes the properties of the solutions affecting T1 so it can be used as a new contrast agents for MRI.
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Keywords: Monte Carlo; MRI; magnetized water; Ising.

- **Introduction**

We can define a Hamiltonian for a system which is dependent on the arrangement of spins on a lattice and from that deduce properties such as Magnetization [1]. Suppose that the Hamiltonian is

$$H = -J\sum_{(ij)} SiSj \; - B\sum_{i} \; Si \qquad (1)$$

Where <ij> means that we sum over the nearest-neighbour pair of spins. This means that the spin at site ij interacts with at i(j ± 1) and (i ± 1)j. The Assuming periodic boundary condition in the model means that every spin will interact with four other spins regardless of their position on the finite lattice. Prefering to figure (1) for better understanding of the proposed system. Here J is the dimensionless interaction strength and B represents the energy involved in the Magnetisation of the lattice and is also dimensionless.

From the Hamiltonian we can calculate the partition function which is

$$Z = \sum_{i} e^{-Hi} \qquad (2)$$

Where we sum over all the particles in the lattice. Then the probability of finding the system in a certain state, denoted S, is

$$P(S) = \frac{e^{-H(S)}}{Z} \qquad (3)$$

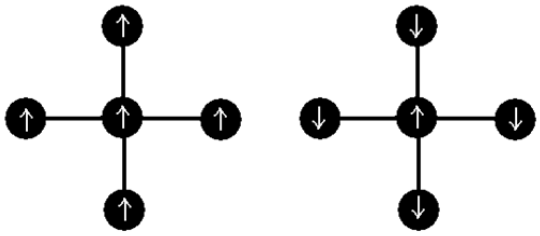

Figure 1. particles on the left all the neighboring particles have the same alignment of spin. and the particle on the right all the neighboring particles have a random spin alignment.

We Quantities such as Magnetization and Energy can be calculated using well known equations from statistical physics. Such as M and E is obtained will give as an estimation of the true value.

$$M = \sum_{S} P(S) \sum_{i} Si \qquad (4)$$

$$E = \sum_{S} P(S)H(S) \qquad (5)$$

It must be pointed out that in the limit of an infinitely large lattice; it is possible to solve the Ising model exactly. If assume that B = 0 the expressions are simplest, it can be seen and we see that the energy and Magnetization are [2,3,4,5].

$$E = -N^2 J \coth(2J)\left[1 + \frac{2}{\pi}\right]\epsilon K_1(k) \quad (6)$$

$$M = \pm N^2 \frac{(1+z^2)^{1/4}(1-6z^2+z^4)^{1/8}}{(1-z^2)^{1/2}} \quad (7)$$

$$k = 2\frac{\sinh(2J)}{\cosh^2(2J)} \leq 1 \quad (8)$$

$$\epsilon = 2\tanh^2(2J-1) \quad (9)$$

$$K_1(k) = \int_0^{\pi/2} \frac{d\phi}{(1-k^2\sin^2\phi)^{1/2}} \quad (10)$$

2. Material and Methods

- **Theoretical model**

To understand the model an implementation that declare some physical behavior of hydrogen Atom in magnetized water like magnetization transfer and magnetization curves for diamagnetic material

Magnetization Curves
Any discussion of the magnetic properties of a material is likely to include the type of graph known as a magnetization or B-H curve

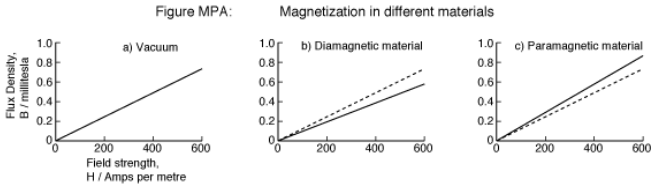

Figure 2. Magnetization Curves

- magnetic field strength as the horizontal axis and the magnetic flux density as the vertical axis.
- Fig. MPA a) is the curve in the absence of any material: a vacuum. The gradient of the curve is $4\pi.10\text{-}7$ which corresponds to the fundamental physical constant $\mu 0$. b) water as Diamagnetic Material[8]

- **Magnetization Transfer**

Macromolecules have a layer of 'bound' water. Since static or slow changing magnetic fields are dominant in the vicinity of macromolecules, the associated hydrogen pool has a very short T2. The correlated fast de-phasing of the transverse magnetization causes this pool of water to be 'invisible'. However, the magnetization of that 'invisible' water pool is transferred to the visible pool of 'free' water via various mechanisms like chemical exchange or cross-relaxation Fig. (3). The term for these processes is called 'magnetization transfer', MT. Cross-relaxation is a special form of dipole-dipole interaction in which a proton on one molecule transfers its spin orientation to that of another molecule. A short T2 or fast de-phasing is synonymous for a broad range of resonance frequencies, whereas a long T2 is indicative of a narrow range. If there are applicable magnetization transfer mechanisms within the tissue, a saturation of the 'invisible' water pool will affect the 'visible' water pool.[7,9]

The T2 is usually so short that this hydrogen pool is not directly observable, and the signal vanishes faster than the ability to acquire some data. The short T2 corresponds to a significant difference in resonance frequencies causing the rapid de phasing. A significant difference in resonance frequencies is a synonym for a very broad resonance of these unobservable protons. The magnetization of these invisible protons can be transferred to the visible "free" water via a chemical exchange or cross-relaxation, which is a special form of dipole–dipole interaction [10].

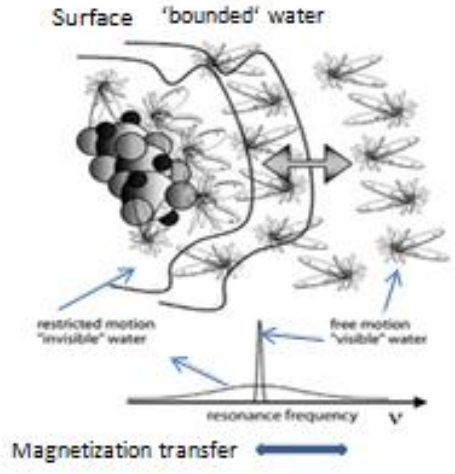

Figure 3. Magnetic Transfer phenomena in Magnetized water

- **The model**

The model is an Ising model in two different cases one with magnetized water(magnetization transfer) , assume the spin is equal 1 or -1,and second case is normal water with random spin, wrote a code in Mat lab that implements the model, a part of the code is borrowed from particularly well written Mat lab code by Tobin Fricke [6].

- **Running the Model**

To begin by creating a square lattice with 128 particles. We also choose a random value between 0 and 1 for the interaction strength and then watch how the system evolves over 1000 steps.. The speed of evolution is controlled by the variable randTol and in this case we decided that randTol= 0.1. A value of 0.1 means that only 10% percent of the originally selected group will have its spin flipped. In essence this parameter tries to mimic the evolution of real systems. Even though a certain particle will have a smaller energy with its spin flipped it doesn't mean that the all the particles in the lattice that follow that criterion will have their spins flipped immediately. We watched the evolution of 1,000 systems and took note of some of the important parameters. We then plot the total energy of the system as a function of interaction, see figure (5).,The computer time needed to finish these computations was approximately 360 seconds in Intel Core 2 Duo CPU 2.67GHZ 4.00GB Ram

- ***practical Method***

Magnetic water phantom Imaging

Two water phantoms used. Each one is constructed of biodegradable latex rubber balloons and filed with 450 ml. one is filled with normal tap water to be used as a reference, where the other is field with magnetic water. Both phantoms are scanned using small body coil of 0.2 Tesla MR (IRIS MATE, Hitachi, Japan). The magnetic water phantom scanned after 4 hours of magnetization figure (4).

The resulted images for both magnetized and non-magnetized water phantoms are quantitatively processed by MATLAP Genetic Algorithms (GAs).

We applied the following signal equation for a repeated spin-echo sequence ,

$$S = k\,(1 - e^{-TR/T1})\,e^{-TE/T2} \qquad (11)$$

This equation is only valid when TR >> TE. In our experiment we used TR= 2700, TE=120, and

$k = 8560*10^{7}$

3. Results

- ***Phantom Imaging analysis***

Quantitative analyses performed using MATLAB Genetic algorithms (GAs) as shown in figure (6) to estimate T1 and T2 for both magnetized and non-magnetized water phantoms based on signal to noise ratio for both images. Table (1) shows imaging parameters in addition to S/N ratio, and results of GAs. We used for both magnetized and non-magnetized images the same calculating parameters as: Function tolerance = 1e-100 ,Generation = 10000 The GAs results shows change in T1, where no changes occurred in T2.

As the positive results obtained from our experiments, the GA in MATLAB show that T1 is Increased to 1.513 s in magnetized phantom instead of 0.672 s in non-magnetized one and T2 did not changed (0.012 s) and S/N increased from 156.3 to 337.5.

The model achieves good confirmation with the expected behavior of magnetization transfer in water although the number of calculated points was not that much. The behavior of the magnetization is plotted in fig.4. The associated energy plot is shown in red.we can note clearly from the model we implement that in non-magnetized water the energy starts from -3 and from magnetized water it starts from -4 indicating changes by 25% also in Magnetized –Energy curves, in magnetic water there is a stable changes in the energy for the system with fixed magnetization ,in no-magnetized water it various so quickly with energy

4. Conclusions

In conclusion, this study clearly indicated a magnetized injection saline and magnetized water as CA enhances MRI. Proved that this technique is a clinically healthy and feasible technique for better diagnosis in MRI Imaging. And we simulate these behavior and results by using Monte Carlo method in Ising model as indicated in many references that Magnetic water is healthy [11-14]

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TABLE 1 WATER PHANTOMS IMAGING PARAMETERS AND RESULTS OF MATLAB GAS

Magnetization	TR	TE	S/N ration	T1	T2
0 Hours	2700	120	**156.3**	672	11
4 Hours	2700	120	**337.5**	1513	12

Non-Magnetized

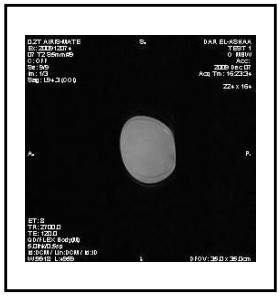

Figure 4. non-magnetized

4hr Magnetized

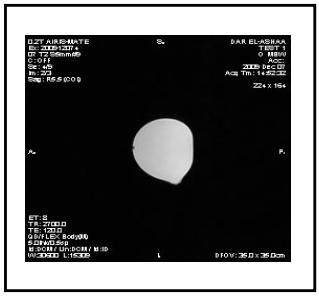

Figure 5. magnetized water

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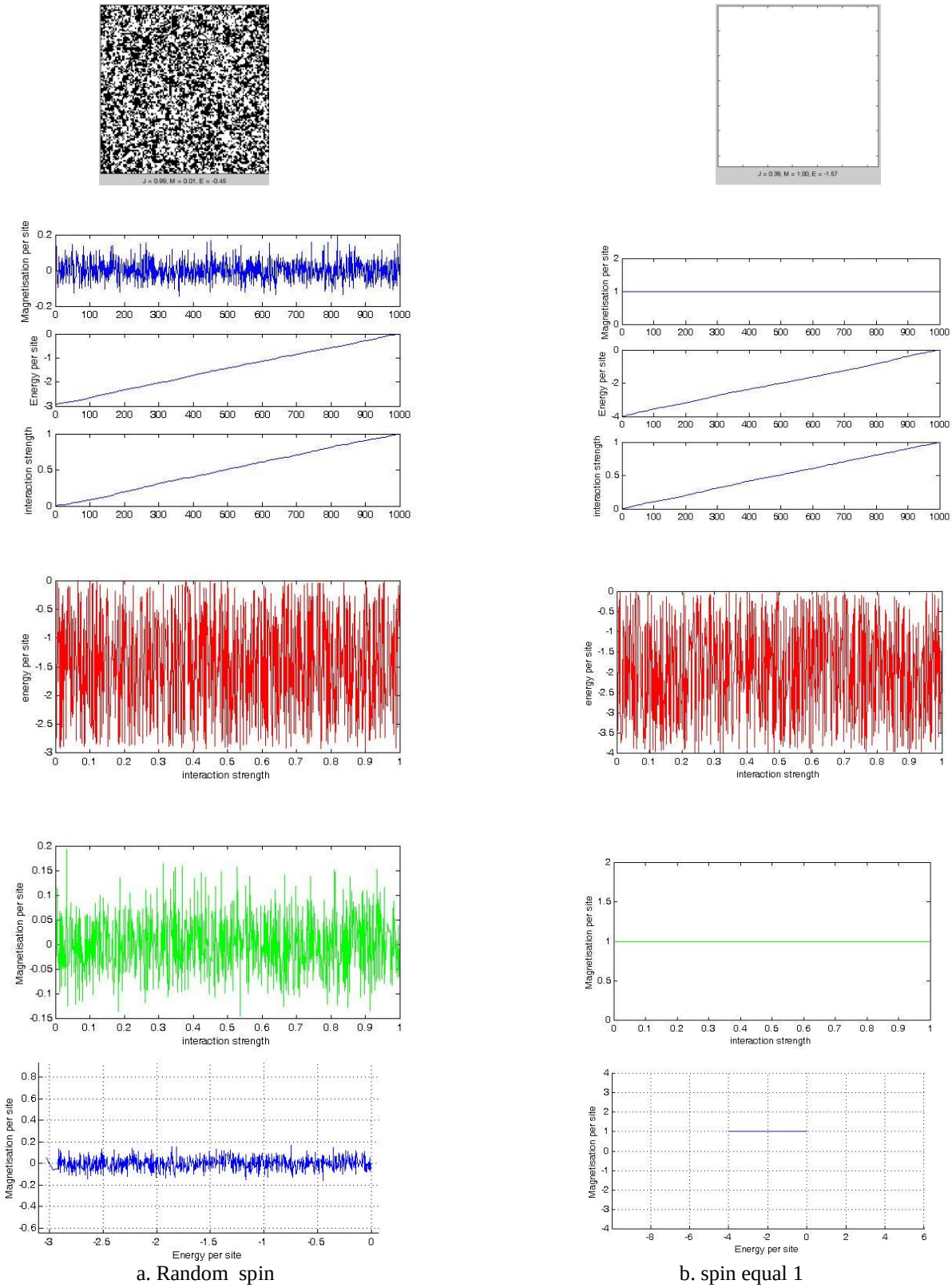

a. Random spin b. spin equal 1

Figure 6. Simulation of the energy behaviour The result fits well with practical expermint

12/21/2010

Multidrug resistant Egyptian isolates of *Acinetobacter baumannii*

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Abstract: The resistance of *Acinetobacter baumannii* to antimicrobial agents is mediated by all of the major resistance mechanisms, including modification of target sites, enzymatic inactivation and active efflux of drugs. Antibiotic susceptibility testing has been performed on fifty-two *A. baumannii* isolates. Twenty isolates have been recovered from patients suffering from wound and burn wound infections attending general surgery, plastic surgery and obstetrics and gynecology departments and thirty-two isolates have been recovered from the environment of these departments. Different mechanisms of antimicrobial resistance have been detected among resistant isolates. Broth dilution method have been used to investigate antimicrobial susceptibility pattern, iodometric method has been used to detect β-lactamase enzymes and polymerase chain reaction has been used to detect $bla_{oxa\text{-}51\text{-}like}$ genes, *aph (3')-VIa* genes and *adeB* gene. Tetracycline was the most effective antimicrobial agent against *A. baumannii*. It has showed high resistance to both of amikacin and meropenem (76.9%), cefipime (80.8%) and both of cephradine and imipenem (96.2%). An extreme resistance to the other antimicrobial agents has been shown by the same organism. β-lactamase enzyme has been detected in β-lactam resistant isolates, $bla_{oxa\text{-}51\text{-}like}$ carbapenemase genes have been detected in carbapenem resistant isolates, *aph (3')-VIa* genes have been detected in amikacin resistant isolates and *adeB* gene have been detected in some multidrug resistant strains. So, resistance to β-lactams, carbapenems and amikacin has been high in *A. baumannii* isolates which has caused appearance of multidrug resistant isolates with different resistance mechanisms like $bla_{oxa\text{-}51\text{-}like}$ genes, *aph (3')-VIa* genes and *adeB* gene.
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Keywords: *A. baumannii*, $bla_{oxa\text{-}51\text{-}like}$ genes, *aph (3')-VIa* genes, *adeB* gene.

1. Introduction

The resistance of *A. baumannii* to antimicrobial agents is mediated by all of the major resistance mechanisms that are known to occur in bacteria. β-lactamases are the most diverse group of enzymes that are associated with resistance, and more than 50 different enzymes, have been identified so far in *A. baumannii*. OXA-51-like carbapenemases are class D β-lactamases which are intrinsic to *A. baumannii* and confer resistance to carbapenems **(1).** Aminoglycoside resistance has been attributed to at least nine distinct modifying enzymes. The emergence of APH (3') enzymes has effectively removed aminoglycosides such as kanamycin and neomycin from clinical use. Resistance to other aminoglycosides such as amikacin and lividomycin serve as the basis for classification into seven distinct classes (I-VII)**.** Another chromosomal system that is typical of *A. baumannii* is the AdeABC efflux system **(2).** Upregulation of AdeABC is so far the only mechanism that has been proven to decrease susceptibility to multiple antimicrobial classes in *A. baumannii*. This work aims to investigate antimicrobial susceptibility pattern among different *A. baumannii* isolates and detect possible mechanisms of resistance in multidrug resistant strains.

2. Material and Methods

Antimicrobial Susceptibility testing. Susceptibility testing was performed on fifty-two *A. baumannii* isolates by using broth dilution method **(3)** in accordance with the guidelines established by EUCAST standards. Twelve different antimicrobial agents were used as follows: Imipenem, meropenem, cephradin, cefipime, amoxycilline/clavulanic acid (Sigma), ciprofloxacin, nalidixic acid, ceftazidme, tetracycline, chloramphenicol, oxacillin (Himedia) and amikacin (Bristol-Myers Squibb).

Detection of β-lactamases . β-lactamase detection was performed using iodometric method **(4).**

Sample preparation for PCR. Isolation of bacterial DNA was performed using DNA extraction kits (Qiagen).

Amplification by PCR. The $bla_{oxa\text{-}51\text{-}like}$ primers (laboratories of The Midland Certified Reagent Company Inc. of Midland) used to amplify $bla_{oxa\text{-}51\text{-}like}$ genes as follows: (5'-TAA TGC TTT GAT CGG

CCT TG-3') as the forward primer and (5'-TGG ATT GCA CTT CAT CTT GG-3') as the reverse primer **(5).** PCRs were carried out using thermal cycler (Techne PROGENE) in 50 µl reaction volumes with 5 µl extracted DNA, 50 pmole of each primer, 25 µl of Go Taq® Green Master Mix (Promega). Conditions were the following: 94°C for 3 min, and then 35 cycles at 94°C for 45 s, at 60°C for 45 s and at 72°C for 1 min, followed by a final extension at 72°C for 5 min. The *aph (3')-VIa* primers (laboratories of The Midland Certified Reagent Company Inc. of Midland) used to amplify *aph (3')-VIa* genes as follows: (5'-ATACAGAGACCACCATAC AGT-3') as the forward primer and (5'-GGACAATCAATAATAGCAAT-3') as the reverse primer **(6).** PCRs were carried out in 50 µl reaction volumes with 5 µl extracted DNA, 50 pmole of each primer, 25 µl of Go Taq® Green Master Mix. Conditions were the following: 94°C for 3 min, and then 30 cycles at 94°C for 1 min, at 55°C for 1 min and at 72°C for 1 min, followed by a final extension at 72°C for 5 min. The *adeB* primers (laboratories of Eurofins MWG Operon) used to amplify *adeB* genes as follows: (5'-GTATGAATTGATGCTGC-3') as the forward primer and (5'-CACTCGTAGCCAATACC-3') as the reverse primer **(7).** PCRs were carried out in 50 µl reaction volumes with 5 µl extracted DNA, 50 pmole of each primer, 25 µl of (Taq PCR Master Mix (2X)) (USB Corporation). Conditions were the following: 94°C for 2 min, and then 35 cycles at 94°C for 30 s, at 55°C for 30 s and at 72°C for 2 min, followed by a final extension at 72°C for 2 min. After amplification, 10 µl of the PCR mixture was analyzed by agarose gel electrophoresis (2% agarose in Tris-borate-EDTA stained with ethidium bromide). The Gene Ruler 100 bp DNA ladder (Fermentas) was used as a DNA size marker.

3. Results

A- Antimicrobial susceptibility testing:

In Table 1, tetracycline was the most effective antimicrobial agent against *A. baumannii*. It showed high resistance to both of amikacin and meropenem (76.9%), cefipime (80.8%) and both of cephradine and imipenem (96.2%). An extreme resistance to the other antimicrobial agents was shown by the same organism.

B- Detection of β-lactamases:

Out of 52 *A. baumannii* strains resistant to different β-lactams tested, 51 strains were β-lactamase producers constituting 98% of the total tested strains (Figure 1).

C- Detection of *bla*$_{oxa\text{-}51\text{-}like}$ genes:

In Figure (2), a group of carbapenem resistant *A. baumannii* was tested for detection of *bla*$_{oxa\text{-}51\text{-}like}$ genes using PCR. It was found that, all tested isolates have been *bla*$_{oxa\text{-}51\text{-}like}$ enzyme producers giving amplicons of 353 bp. size.

D- Detection of *aph (3')-VIa* genes :

A group of amikacin resistant *A. baumannii* was tested for detection of *aph (3')-VIa* genes using PCR. It was found that, all resistant isolates were *aph (3')-VIa* enzyme producers giving amplicons of 234 bp. Size, (Figure 3).

E- Detection of *adeB* gene:

In Figure (4), a group of multidrug resistant *A. baumannii* isolates was tested for detection of *adeB* genes using PCR. It was found that some of the tested isolates were *adeB* gene positive giving amplicons of 979 bp. size and the other *A. baumannii* isolates were *adeB* gene negative.

Table 1: Incidence of antimicrobial resistance among *A. baumannii* isolates (52).

Antimicrobial agents	Number of resistant strains (%)*
Imipenem	50 (96.2%)
Meropenem	40 (76.9%)
Ciprofloxacin	52 (100%)
Nalidixic acid	52 (100%)
Chloramphenicol	52 (100%)
Tetracycline	0 (0%)
Amikacin	40 (76.9%)
Cefipime	42 (80.8%)
Oxacillin	52 (100%)
Amoxycilline/ clavulanic acid	52 (100%)
Cephradine	50 (96.2%)
Ceftazidime	52 (100%)

* Percentage was correlated to the no. of *A. baumannii* isolates.

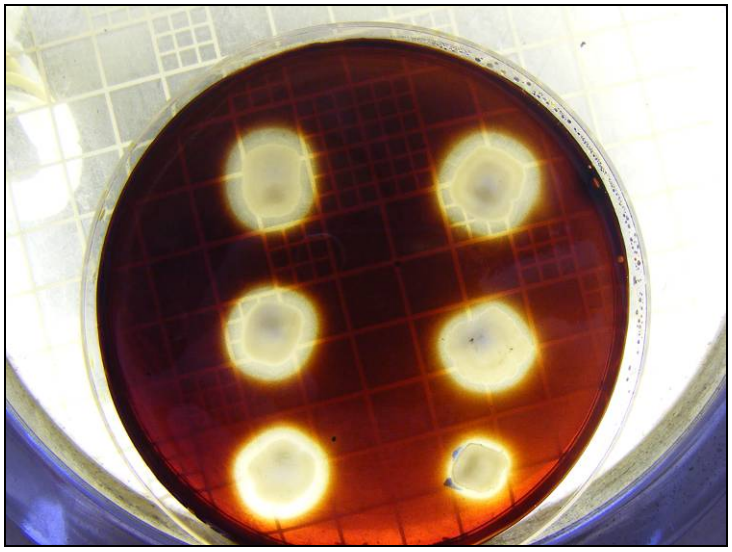

Figure (1): β-lactamase production by *A. baumannii isolates*.
The bottom right of the picture: control strain, The other strains: β-lactamase +ve strains

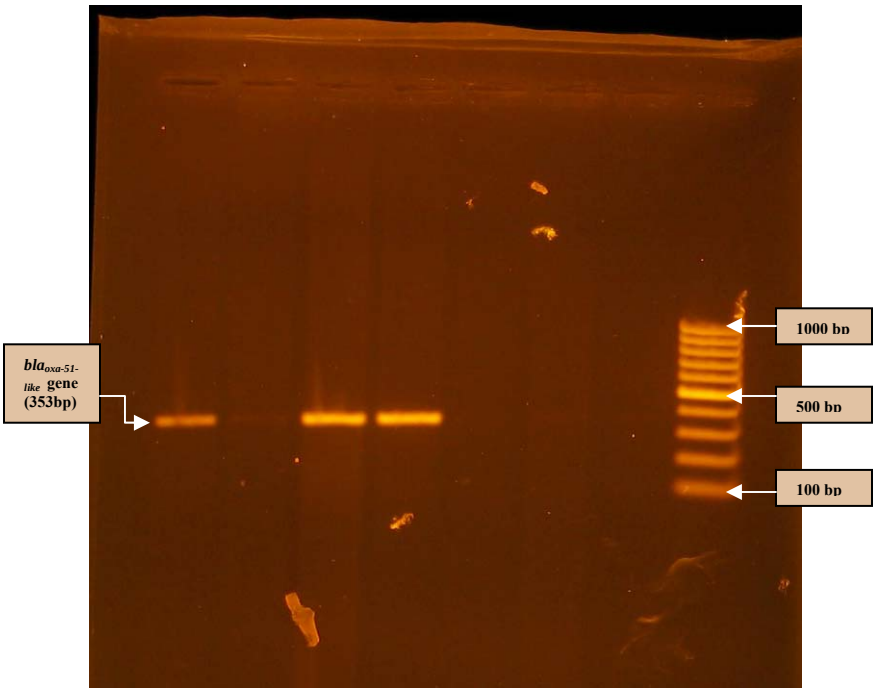

Figure (2): PCR results to amplify fragments of $bla_{oxa-51-like}$ genes in different carbapenem resistant *A. baumannii* isolates.
From right to left: Lane 1: DNA ladder, lanes 2 and 3: two control *E. coli* isolates, lane 4: control Klebsiella isolate, lanes 5, 6, 7 and 8: four carbapenem resistant *A. baumannii* isolates.

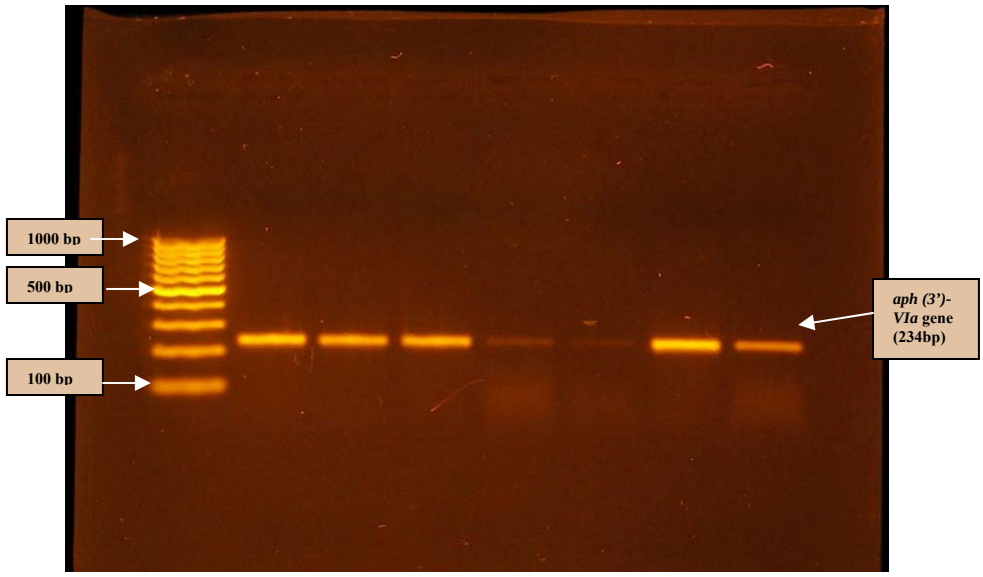

Figure (3): PCR results to amplify fragments of *aph (3')-VIa* genes in different amikacin resistant *A. baumannii* isolates.
From left to right: Lane 1: DNA ladder, lanes 2, 3, 4, 5, 6, 7 and 8: seven amikacin resistant *A. baumanni* isolates.

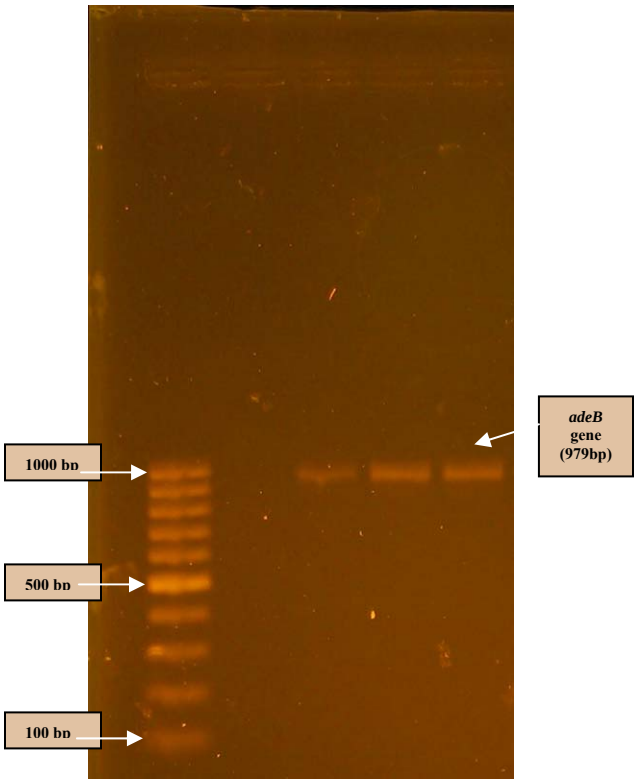

Figure (4): PCR results to amplify fragments of *adeB* genes in multidrug resistant *A. baumannii* isolates.
From left to right: Lane 1: DNA ladder, lane 2: *adeB* negative *A. baumannii* isolate, lanes (3-5): *adeB* positive *A. baumannii* isolates.

4. Discussion

Antimicrobial Susceptibility Testing:

A. baumannii isolates have showed high resistance to imipenem, meropenem, cefipime, ceftazidime, ciprofloxacin and β-lactam combination (100%), amikacin (98.8%) and no resistance to tigecycline. These results agree with the results obtained in our study for tetracycline derivative (tigecycline) (most effective), ceftazidime, ciprofloxacin and β-lactam combination, but it showed higher resistance with imipenem, meropenem, cefipime and amikacin **(8).**

A. baumannii have showed high resistance to ceftazidime (94.6%), tetracycline (81.2%), meropenem (80.4%), imipenem (78.6%) and ciprofloxacin (76.8%) and low resistance to cefipime (34.8%). These results are in agreement with the results obtained in the present study for ceftazidime, meropenem, imipenem and ciprofloxacin (high resistance) but it indicates higher incidence of resistance to tetracycline and lower incidence of resistance to cefipime **(9).**

It has been found that *A. baumannii* isolates have showed high resistance to cefipime (79.1%), meropenem and imipenem (70.5%), amikacin (61.2%) and low resistance to tigecycline (7.9%) **(10).** These results agree with the results obtained in our study.

It has been reported that *A. baumannii* strains had high resistance to ciprofloxacin (90.53%), cefipime (82.30%), imipenem (81.61%) and meropenem (75.31%) and low resistance to amikacin (33.33%). Our results showed the same behaviour with ciprofloxacin, cefipime, imipenem and meropenem (high resistance) but indicates higher resistance to amikacin **(11).**

It has been found that *A. baumannii* strains had high resistance to ciprofloxacin (98%), amikacin (96%), meropenem (95.5%) and imipenem (87.8%). These results are in agreement with the results obtained in the present study for ciprofloxacin, amikacin, meropenem and imipenem **(12).**

A. baumannii showed high resistance to ceftazidime (83.3%) and amikacin and ciprofloxacin (77.8%) and low resistance to meropenem (11.1%) and imipenem (9.1%). Our results showed the same behaviour with ceftazidime, amikacin and ciprofloxacin (high resistance) but indicated higher resistance to meropenem and imipenem **(13).**

Detection of β-lactamases and *bla*$_{oxa\text{-}51\text{-}like}$ genes:

Among imipenem-susceptible and resistant *A. baumannii* which were screened by PCR for different β-lactamases. The *bla*$_{oxa\text{-}51\text{-}like}$ gene was the only one detected, even in imipenem-susceptible strain **(14).** It has been reported that among *A. baumannii* isolates with *bla*$_{oxa\text{-}51\text{-}like}$ as sole carbapenemase gene, imipenem and/or meropenem resistance was associated only with isolates in which ISAba1 was upstream of *bla*$_{oxa\text{-}51\text{-}like}$, suggests that ISAba1 is providing the promoter for this gene **(1).** Oxa-51-like subgroup enzyme may be involved in the expression of carbapenem resistance under certain circumstances. Also, all imipenem-resistant *A. baumannii* isolates were positive for carbapenemase production and negative for metallo β-lactamase. They all possessed the encoding gene for an intrinsic oxa-51-like carbapenemase and an acquired oxa-23-like carbapenemase **(15).**

Detection of *aph (3')-VIa* genes:

All 16 clinical amikacin resistant *A. baumannii* isolates had positive PCRs with primers specific for the amplification of the *aph (3')-VIa* gene which confirms the contribution of the *aph (3')-VIa* gene to the incidence of amikacin resistance in *A. baumannii* **(6).** 97% of *A. baumannii* isolates that are amikacin resistant contained the phosphotransferase gene aphA6 (*aph (3')-VIa*) **(16).** Among 106 multidrug resistant clinical *A. baumannii* strains from hospitals in the Czech Republic and other European countries, aph A6 gene was predominant in 55 strains representing (52%) **(17).** It has been illustrated that among 49 clinical isolates of multidrug resistant *A. baumannii* identified at a tertiary medical center in Pennsylvania, the *aph (3')-VIa* gene was detected in 3 isolates representing (6.1%) **(18).**

Detection of *adeB* efflux pump gene:

All tested 39 multidrug resistant Acinetobacter strains had the *adeB* gene and disruption of the *adeB* gene has greater effect on resistance to meropenems than *adeA* gene in Acinetobacter spp. isolated from university Malaya medical centre **(19).** High distribution of *adeB* (91.8%) gene in multidrug resistant *A. baumannii* isolates from the three military hospitals in China has been observed **(12).** The majority of the *A. baumannii* isolates (75%) that generally display high-level multidrug resistance were positive for *adeSR-adeABC*, suggesting a potential linkage between these genes and multidrug resistance **(20).**

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Evaluation of the effect of three different pesticides on *Azolla pinnata* growth and NPK uptake

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Abstract: Three pesticides of common use in rice fields in Egypt were used in the present work. This study was devoted to investigate the effects of different concentrations of the insecticide furadan, fungicide hinosan and herbicide saturn on the growth and NPK uptake of the aquatic fern *Azolla pinnata,* which is recommended to be applied as a biofertilizer in rice. In this respect, the results obtained showed variable effects of the three pesticides under study. Furadan and hinosan showed positive effects since each increased the growth rate of *A. pinnata* at lower concentrations (0.001, 0.002 ppm) and consequently increased its NPK content. Maximum dinitrogenase activity was also generally obtained at 0.002 ppm furadan, throughout the different incubation periods. Nitrogen, phosphorus and potassium uptake was generally increased with increasing the incubation period of the applied furadan and hinosan, at all concentrations. The highest NPK uptake by *A. pinnata* was obtained with the medium concentration (0.002 ppm) of both pesticides after 20 and 25 days of incubation. On the other hand, saturn generally showed inhibitory effects on the growth, N_2- fixation and NPK uptake even at lowest concentration (0.001 ppm).
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Keywords: *Azolla pinnata,* fungicides, insecticides, herbicides saturn, hinosan, furadan, growth, dinitrogenase activity, uptake of nitrogen, phosphorus, potassium.

1. Introduction

Azolla– Anabaena symbiosis is an important N_2-fixing system between an eukaryotic fern and a prokaryotic cyanobacterium. This host-symbiont combination is exploited as a biofertilizer for many agricultural crops (Lillian 2000; Pabby et al., 2003 and Abd El- Rasoul et al., 2004). Like most plants, *Azolla* requires the macro-and micronutrients which are essential for normal growth. Nitrogen, phosphorus, potassium, calcium and magnesium are very important and produce marked effects on the fern growth (Arrora et al., 2003). It was found that application of *Azolla* appreciably improved soil fertility by increasing its total nitrogen, organic carbon and available phosphorus (Singh and Singh, 1990; Jeyabal and Kuppuswamy, 2001 and Ghoudhary and Kennedy, 2004). Investigations showed that *Azolla* is also a promising plant to be applied in controlled ecological life support systems (Xiaofeng et al., 2008). Agriculture is almost dependent on chemical pesticides (Greaves et al., 1988). However, pesticides are observed to exert determinable effects on microbial processes, which play an important role in plant growth, crop productivity and soil fertility (Nayak and Rajamamoban, 1982). The carbamates, like organochlorine insecticides, have received a moderate amount of attention in relation to their influence on cyanobacteria in paddy field ecosystems

(Hammouda, 1999). Carbofuran (furadan) is a systematic insecticide which means that it is absorbed by the plant roots and distributed to all parts of the plant and it is a member of the carbamate family which inhibits the enzyme cholinesterase by forming carbofuran -ACHE complex (Chauhan et al., 2000 and Class Resourses, 2009).

Singh et al. (1982) and Watanabe (1986) showed that mixing small amount of the insecticide carbofuran (furadan) with the *Azolla* inoculums effectively controlled most of the insects attacking *Azolla* and so increased its growth. However, addition of lower concentrations of lindane increased growth and N_2- fixation (Singh et al., 1982). The authors added that further increase of these concentrations decreased growth and N_2 fixation with all tested *Azolla* species. On the other hand, Ismail et al. (1995) found that carbofuran significantly increased *A. pinnata* chlorophyll content and dinitrogenase activity but did not affect its growth.

Rice blast is one of the most destructive diseases in rice plant (Savary et al., 2000) and is mainly controlled by application of the fungicide henosan (O- Ethyl- S- S- diphenyle-

dithiophosphate). It is considered as one of the organophosphate group (Madhaiyan et al., 2006). The phosphorothiolate (hinosan), edifenphos and iprobenfos fungicides have been used to control rice blast in rice cultivating areas (Kim et al., 2008 and Kim and Kim, 2009). On the other hand, the inhibition on the growth of *Gluconacetobacter diazotrophicus* showed no significant differences with the variation of the added doses of dithane and hinosan to the growth media (Madhaiyan et al., 2006).

Every year, major losses in paddy occur due to heavy weed infestation. There are various herbicides to control weeds but most of them have toxic residues in different parts of the rice plant (Aktar et al., 2007). Thiobencarb (S- 4-chlorobenzyle diethyl thiocarbamate) had been extensively used in modern agricultural practices (Xia, 2004). It is mainly used as a pre or post emergent herbicide (Aktar et al., 2007). One of the currently used formulations of benthocarb is saturn 50 EC. This compound is absorbed by the root systems of the herbs, translocates to the meristem and inhibits protein synthesis (Matsuo and Shibayama, 2002). On the other hand, sensitivity of cyanobacteria to herbicides varies according to the species (Sabuter and Carrasco, 1996) and the kind of herbicide but, in general, they are quite sensitive to herbicides (Irisarri et al., 2001).

Azolla growth was influenced by various herbicides. Butachlor (mashete) and benthiocarb (saturn) when applied at high concentrations showed maximum inhibitory effect on the *Azolla* growth and N_2-fixation (Singh and Mishra, 1982). Zarger and Dar (1990) also suggested that thiobencarb had inhibitory effect on nitrogen fixation and heterocyst formation in a mixed culture of *Anabaena*, *Nostoc* and *Oscillatoria.* Battah et al. (2001) added that saturn inhibited protein synthesis, which exert many secondary effects on growth. Xia (2004) added that biomass yield, protein content and photosynthetic rate were reduced only at high thiobencarb concentrations. Lales and Marte (1986) found that propanil and butachlor were most toxic to all treated ferns. On the other hand application of 2, 4-Dina stimulated the growth of *Azolla* (Singh et al., 1988).

Aim of work:

The use of insecticide furadan, fungicide hinosan and herbicide saturn has become of common practice in rice cultivation to kill the insects in culture, reduce fungal infections, and to minimize the cost involved in weeding. Algae have frequently been the subject of this investigation because of their importance as primary producers in fresh water systems. Therefore, studying the effects of elevated concentrations of these pesticides on the growth of *Azolla* is particularly important for recording its detrimental effect on different growth parameters, nitrogen- fixation and NPK uptakes by *Azolla pinnata*.

2. Material and Methods

Materials:

Azolla pinnata used in the present investigation was kindly provided by the Agricultural Microbial Department, Soils, Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC), Giza, Egypt.

The insecticide furadan (carbofuran; 2,3 dihydro- 2, 2- dimethylbenzofuran 7-methylcarbonate), the fungicide hinosan (O - Ethyl-S,S- diphenyl phosphorodithioste) and the herbicide saturn (S- chlorobenzyl- N, N- diethyl thiocarbamate) were kindly provided from the Central Laboratory of Pesticides, ARC, Dokki, Cairo, Egypt.

Methods:

The present experiment was carried out in the Botanical garden of the Faculty of Science, Ain Shams University, Cairo, Egypt.

Propagation of *Azolla:*

According to El- Shahat (1988), ten g *Azolla pinnata* were grown in plastic pots (32 cm in diam. and 15 cm dep.), each containing 1 Kg soil in 3 liters tap water then kept in a greenhouse till *Azolla* covered the entire water surface. *Azolla* was collected and incorporated in 0.01 mercuric chloride for 1 min. and washed gently in running tap water for several times, using a screen of 0.2 mesh, and then air dried on tissue paper for 30 min. The collected fronds were used as an inoculum for further experiments.

The effects of the insecticide furadan, the fungicide hinosan, and the herbicide Saturn on the *Azolla* growth were tested. For this purpose, modified Yoshida medium (Yoshida et al., 1976) was prepared to contain increased concentrations of $NaH_2PO4.H_2O$,$CaCl_2$, K_2SO_4 and $MgSO_4.7H_20$ (40 ppm) and trace elements 1 ml per litter. Thirteen sets of plastic pots (14 x 7 cm) were used; each pot contained 750 ml Yoshida medium. Different concentrations (0.00, 0.001, 0.002, 0.003, 0.004 ppm) of each pesticide (furadan, hinosan and saturn) were added to the Yoshida liquid medium and inoculated by one gram fresh *Azolla*. Each concentration was represented by one set and every set consists of 3 replicates for each incubation period. The pots were incubated under normal condition of light (18/6 hr.)

and temperature of 25 ºC ±2 at day time and 18 ºC ±2 at night and air humidity of about 70% for 25 days.

The Yoshida medium was changed every five days to get constant concentrations of minerals and pesticides used throughout the experiment. Developed *Azolla* culture was periodically sampled after 5, 10, 15, 20 and 25 days. Harvested *Azolla* fronds were washed by deionized water and placed under shade between two thick layers of blotting papers for approximately 1 hour before determining fresh and dry weights, doubling time, dinitrogenase activity and NPK uptake.

Calculation of doubling time:

Growth rate of *Azolla* in terms of doubling time was calculated using the following equation according to Aziz and Watanabe (1983).

Doubling time = t/r, whereas:

t = the duration of *Azolla* growth.

r = log wt /wo / 0.301

Wt = weight of *Azolla* at time t,

W0 = weight of *Azolla* at zero time i.e. weight of inoculum.

Acetylene reduction assay:

About 0.5 g fresh weight of *Azolla* of each treatment was incubated under 10% C_2H_2 in air of 500 ml flask, fitted with serum caps and containing 100 ml amended culture media.

The incubation conditions and analysis of ethylene produced were adopted as described by Kitoh et al. (1993).

NPK content:

NPK content of *Azolla* was estimated in dried plant material. Nitrogen was determined using microkjedahl method according to Black et al. (1965), phosphorus spectrophotometerically according to Olsen and Sammers (1982) and total potassium according to the method described by Jackson (1958).

Statistical analysis:

The individual data sets were subjected to the least significant differences at $p<0.05$ as calculated by Gomez and Gomez (1984).

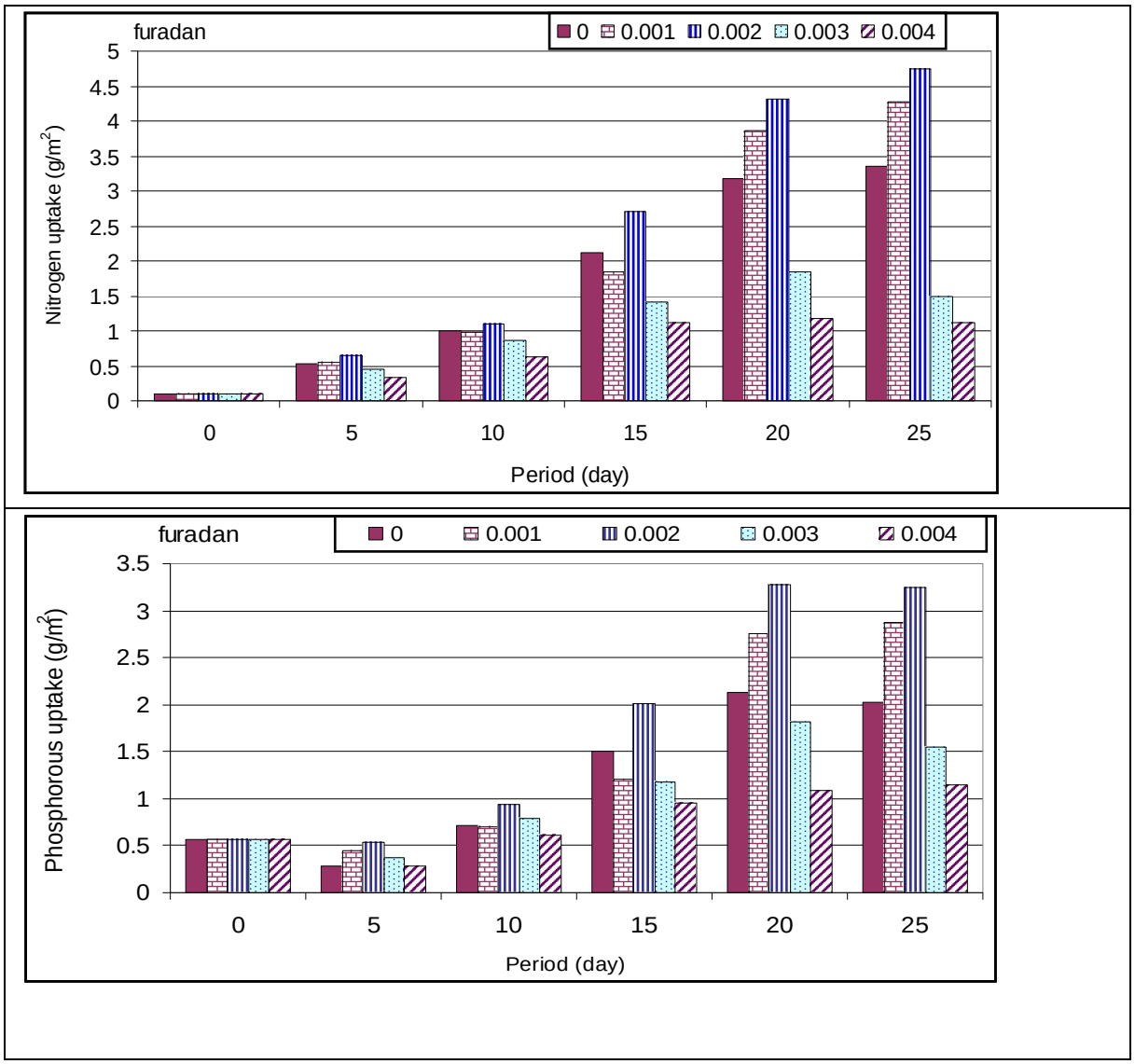
3. Results and Discussion

The different pesticides used in the present work showed varying effects on *Azolla pinnata.* A range of concentrations (0.00, 0.001, 0.002, 0.003 and 0.004 ppm) of furadan, hinosan and saturn were used to verify the optimal level which can be used without affecting the viability of Azolla.

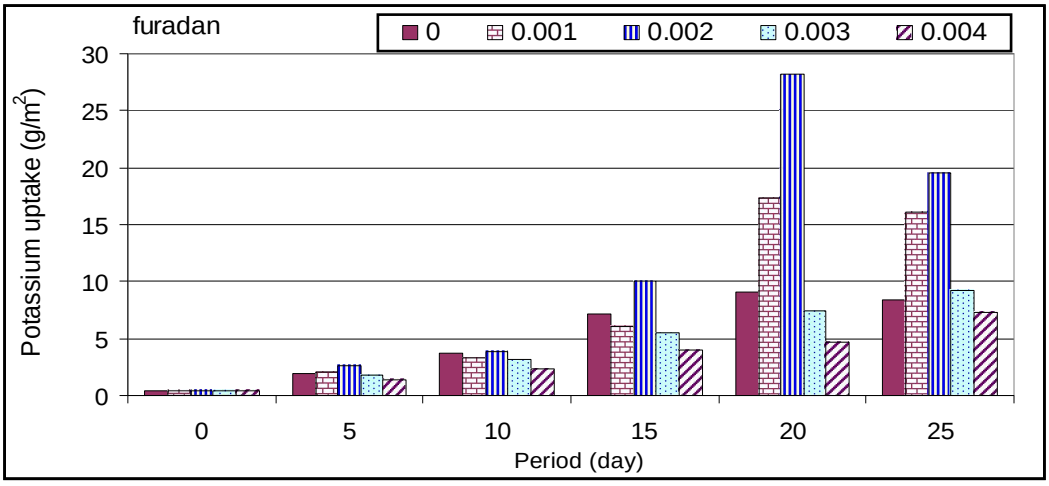
The data in Tables (1&2) showed that the fresh and dry weights of *A. pinnata* were optimal after 20 days incubation period at 0.002 ppm furadan and hinosan. A similar trend was found by Watanabe (1982) and Singh et al. (1984) who reported that mixing small amount of insecticide with *Azolla* inoculum effectively controlled most of the attacking insects and increased plant growth and N_2-fixation, while higher concentration decreased growth and N_2- fixation. On the other hand, Singh and Sethunathan (1999) found that *Azolla* could utilize carbofuran (furadan) as a sole source of carbon and nitrogen.

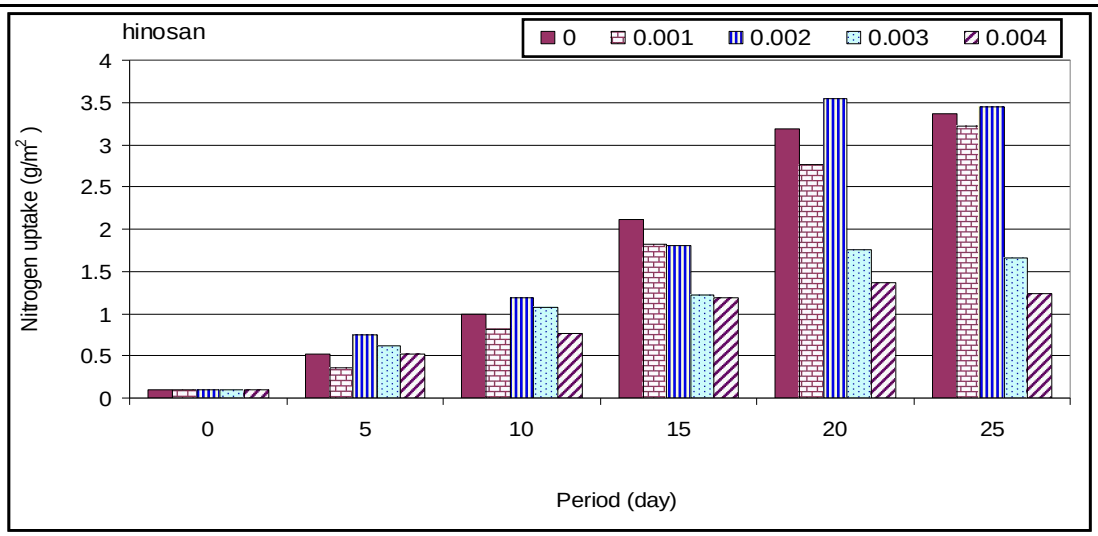
A. pinnata appeared to be sensitive to all concentrations of saturn. This observation was derived from that fronds grown in the control showed higher accumulation of fresh and dry weights than those grown in different concentrations of saturn at 5 days up to 25 days of incubation (Tables:1& 2). These results agreed with those of Lales and Marte (1986) who found that the fresh weight of *Azolla* was influenced by various herbicides. Singh and Mishra (1982) observed that benthiocarb (saturn) showed maximum inhibitory effect on *Azolla* growth and N_2-fixation. It was found that saturn has an inhibitory effect on photosynthetic CO_2 assimilation (Battah et al., 2001) and inhibited protein synthesis (Xia, 2004), which could be due to disturbances in nitrogen metabolism and photosynthetic activity (Battah et al., 2001) or due to an increase in protease activity (Bhunia et al., 1991). Such effects might exert many secondary effects on growth.

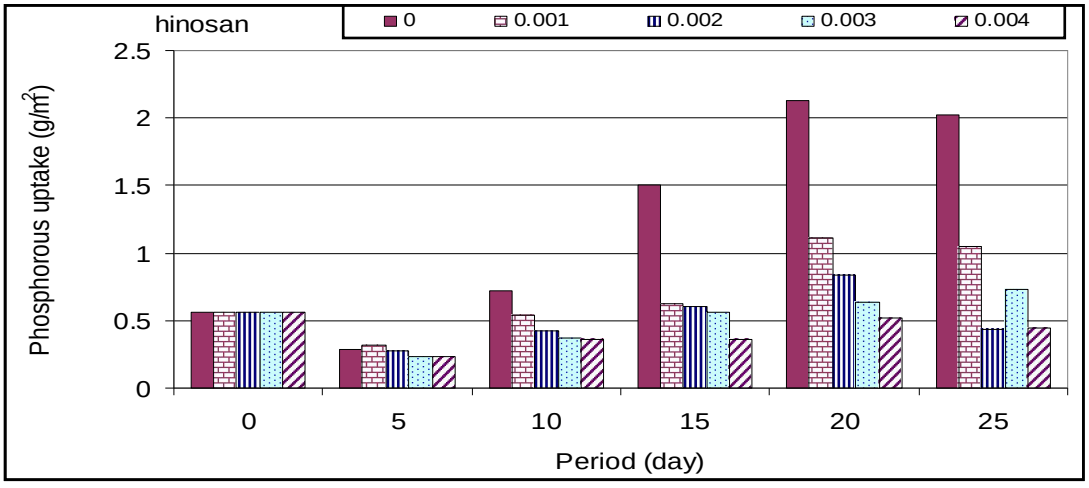
Hopkins (1998) stated that chemical herbicides have become an important management tool, but their value as a labor saving device must be carefully weighted against potentially harmful side effects.


furadan
0
0.001
0.002
0.003
0.004
Nitrogen uptake (g/m2)
Period (day)
0
5
10
15
20
25
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5

furadan
0
0.001
0.002
0.003
0.004
Phosphorous uptake (g/m2)
Period (day)
0
5
10
15
20
25
0
0.5
1
1.5
2
2.5
3
3.5


furadan
0
0.001
0.002
0.003
0.004
Potassium uptake (g/m2)
30
25
20
15
10
5
0
0
5
10
15
20
25
Period (day)


hinosan
0
0.001
0.002
0.003
0.004
Nitrogen uptake (g/m2)
4
3.5
3
2.5
2
1.5
1
0.5
0
0
5
10
15
20
25
Period (day)


hinosan
0
0.001
0.002
0.003
0.004
Phosphorous uptake (g/m2)
2.5
2
1.5
1
0.5
0
0
5
10
15
20
25
Period (day)

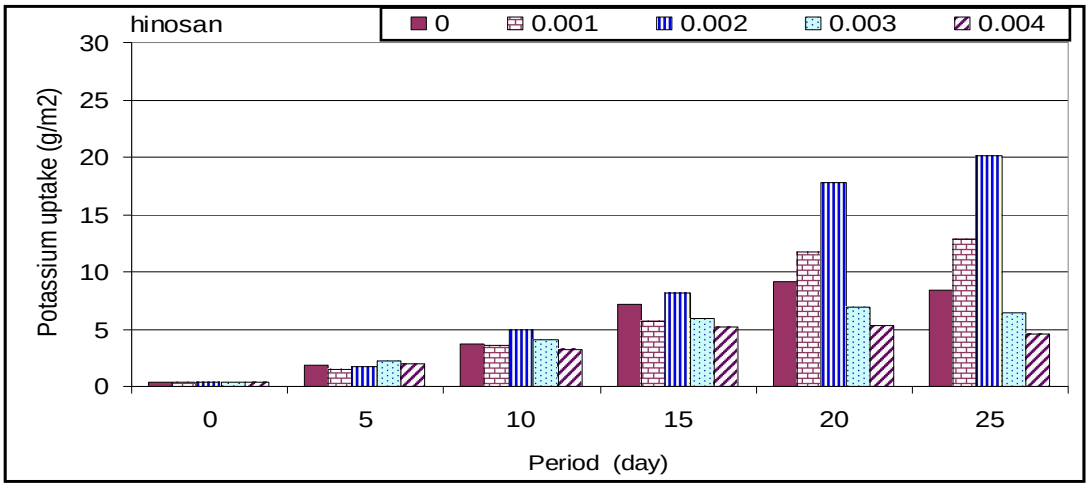
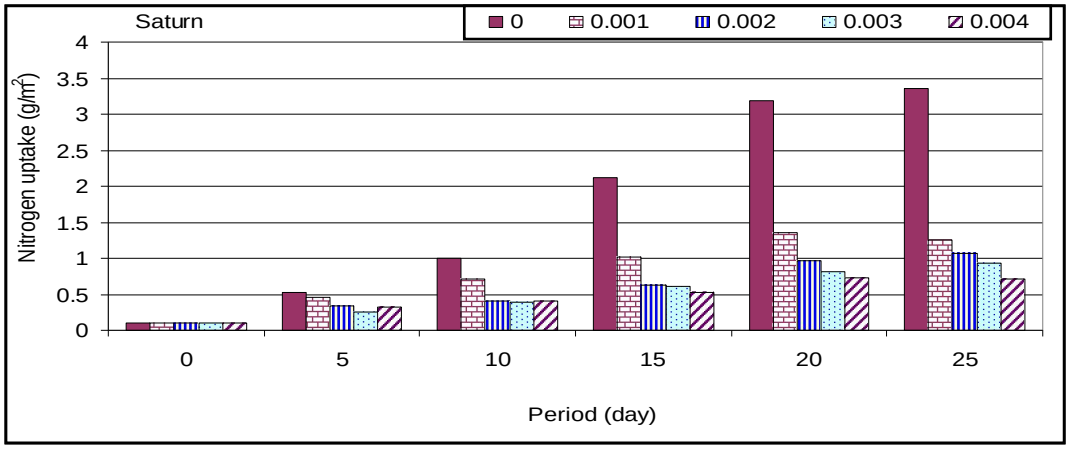
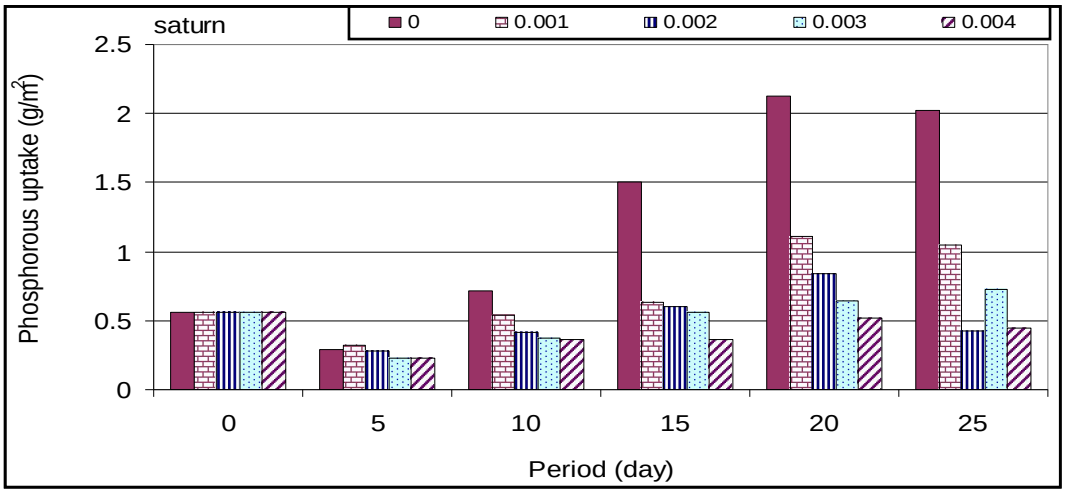


Fig. (2): Effect of different concentrations of hinosan on nitrogen, phosphorous and potassium uptake (g/m^2) by *Azolla pinnata*





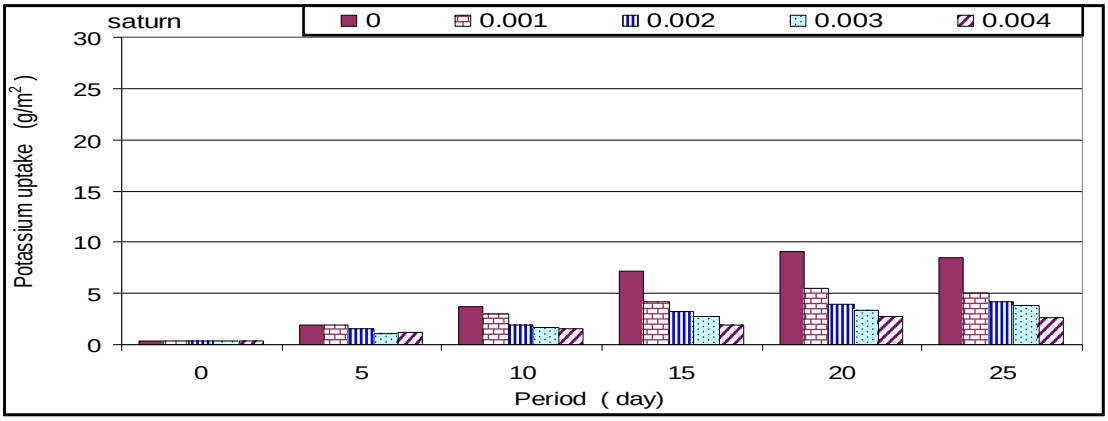


Fig. (3): Effect of different concentrations of Saturn on nitrogen, phosphorous and potassium uptake (g/m^2) by *Azolla pinnata*

Table (1) : Effect of different concentrations of furadan, hinosan and saturn on *Azolla pinnata* fresh weight (g/m^2).The values listed are the means of 3 replicated ± SD (stander deviation)

Treatment / Period (days)	control	furadan				hinosan				saturn			
	Concentration (ppm)												
	0.00	0.001	0.002	0.003	0.004	0.001	0.002	0.003	0.004	0.001	0.002	0.003	0.004
0	**90.90±**	**90.90±**	**90.90±**	**90.90±**	**90.90±**	**90.90±**	**90.90±**	**90.90±**	**90.90±**	**90.90±**	**90.90±**	**90.90±**	**90.90**
5	**586. 32** ±7.41	**404.54** ±11.41	**479.90** ±6.92	**346.65** ±7.89	**289.69** ±5.95	**270.90** ±19.90	**333.63** ±4.25	**423.63** ±14.96	**390.00** ±5.05	**378.14** ±7.41	**307.69** ±15.06	**227.24** ±41.66	**290.88** ±24.05
10	**707.20** ±39.04	**592.73** ±22.98	**667.81** ±12.37	**560.90** ±0.98	**432.72** ±15.37	**596.36** ±13.69	**745.45** ±9.50	**667.27** ±7.65	**562.73** ±8.17	**569.96** ±12.63	**354.51** ±34.41	**345.41** ±32.77	**358.51** ±38.43
15	**1221.11** ±20.33	**934.54** ±4.27	**1357.27** ±17.46	**870.00** ±1.00	**692.72** ±5.84	**966.00** ±10.52	**953.72** ±6.18	**740.00** ±5.46	**737.27** ±4.21	**752.58** ±16.37	**532.70** ±73.27	**527.22** ±30.46	**457.23** ±8.75
20	**1532.33** ±40.09	**1692.1** ±8.10	**1830.02** ±19.98	**1079.0** ±1.50	**712.41** ±8.44	**1200.0** ±18.36	**1632.7** ±8.48	**1020.40** ±13.21	**830.90** ±6.95	**987.81** ±29.22	**713.57** ±44.12	**636.3** ±81.30	**620.85** ±29.51
25	**133.63** ±30.87	**1553.4** ±18.41	**1655.15** ±15.05	**898.18** ±1.07	**687.27** ±1.64	**1279.0** ±5.80	**1559.0** ±18.33	**923.69** ±6.20	**797.27** ±12.54	**855.40** ±18.18	**756.31** ±37.56	**719.04** ±13.03	**573.58** ±8.20
L.S.D. at 0.05 P													
Conc. x time		97.741				41.590				96.621			

Table (2) : Effect of different concentrations of furadan, hinosan and saturn on *Azolla pinnata* dry weight (g/m^2). The values listed are the means of 3 replicated ± SD (stander deviation)

Treatment / Period (days)	control	furadan				hinosan				saturn			
	Concentration (ppm)												
	0.00	0.001	0.002	0.003	0.004	0.001	0.002	0.003	0.004	0.001	0.002	0.003	0.004
0	**5. 09**	**5. 09**	**5. 09**	**5. 09**	**5. 09**	**5. 09**	**5. 09**	**5. 09**	**5. 09**	**5. 09**	**5. 09**	**5. 09**	**5. 09**
5	**22. 64** ±1.34	**23.05** ±0.23	**26. 83** ±0.39	**19. 41** ±0.44	**14. 50** ±0.70	**15. 17** ±1.12	**18. 68** ±0.23	**23.72** ±0.83	**21. 84** ±0.27	**21. 17** ±1.81	**17. 23** ±0.72	**12.73** ±2.33	**16. 29** ±1.35
10	**39. 85** ±2.49	**33.19** ±0.23	**37. 39** ±0.66	**31. 42** ±0.06	**24. 23** ±0.86	**33. 39** ±0.78	**41.74** ±0.54	**37. 36** ±0.44	**31. 50** ±0.48	**31. 92** ±0.72	**19. 85** ±1.93	**19. 34** ±1.84	**20. 08** ±2.15
15	**68. 38** ±1.39	**52.33** ±0.08	**77.24** ±0.80	**48.72** ±0.03	**38. 23** ±0.33	**54. 09** ±0.59	**53. 40** ±0.35	**41. 44** ±0.28	**41. 29** ±0.25	**42.14** ±0.92	**29. 83** ±4.11	**29. 52** ±1.71	**25. 60** ±4.33
20	**85.14** ±1.83	**94.79** ±0.43	**102. 49** ±1.10	**60. 46** ±0.08	**39. 89** ±0.48	**67. 20** ±0.99	**91. 43** ±0.44	**57.14** ±0.75	**46. 51** ±0.36	**55. 32** ±1.62	**39. 95** ±2.47	**35. 63** ±4.65	**34.76** ±1.67
25	**74. 68** ±2.12	**86. 99** ±0.90	**92. 68** ±0.85	**50. 09** ±0.09	**38. 48** ±0.09	**71. 62** ±0.30	**87. 31** ±1.06	**51.72** ±0.35	**40. 90** ±0.58	**47. 90** ±1.02	**42. 35** ±2.11	**40. 26** ±1.47	**32.12** ±0.46
L.S.D.at 0 .05 P Conc. x time		*4.151*				2.013				3.465			

Table (3): Effect of different concentrations of furadan, hinosan and Saturn on doubling time (day) of *Azolla pinnata*. The values listed are the means of 3 replicated ± SD (stander deviation)

Treatment / Period (days)	Control	furadan				hinosan				saturn			
	Concentration (ppm)												
	0.00	0.001	0.002	0.003	0.004	0.001	0.002	0.003	0. 004	0.001	0.002	0.003	0.004
0	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
5	**2.3** ± 0.10	**2.3** ± 0.11	**2.1** ±0.06	**2.6** ± 0.1	**3.0** ±0.26	**3.2** ± 0.15	**2.7** ±0.12	**2.3** ±0.1	**2.4** ±0.06	**2.4** ±0.15	**2.8** ±0.10	**3.9** ±0.7	**3.0** ±0.26
10	**3.4** ± 0.10	**3.7** ± 0.06	**3.5** ± 0.10	**3.8** ± 0.2	**4.4** ±0.10	**3.7** ±0.06	**3.3** ±0.06	**3.5** ±0.1	**3.8** ±0.10	**3.7** ±0.06	**5.1** ±0.38	**5.2** ±0.4	**5.1** ±0.40
15	**4.4** ± 0.06	**4.5** ± 0.10	**3.8** ± 0.17	**4.6** ± 0.1	**5.1** ± 0.38	**4.4** ± 0.06	**4.4** ± 0.06	**4.9** ± 0.1	**4.9** ± 0.10	**4.9** ± 0.00	**5.9** ± 0.45	**5.9** ± 0.2	**6.6** ± 0.10
20	**6.0** ± 0.17	**4.7** ± 0.10	**4.6** ± 0.10	**5.6** ± 0.1	**6.8** ± 0.06	**5.4** ± 0.15	**4.8** ± 0.15	**5.7** ± 0.1	**6.2** ± **0.15**	**5.8** ± 0.06	**6.7** ± 0.23	**7.1** ± 0.5	**7.2** ± 0.15
25	**7.9** ± 0.23	**6.1** ± 0.17	**5.9** ±0.45	**7.6** ±0.1	**8.6** ±0.10	**6.6** ± 0.10	**6.1** ± 0.17	**7.5** ± 0.2	**8.4** ± 0.05	**7.7** ± 0.06	**8.2** ± 0.20	**10.1** ± 0.4	**9.4** ± 0.06
L.S.D. at 0 .05 P Conc. x time		0.257				0.143				0.239			

Conc. x time

Table (4) : Effect of different concentrations of furadan , hinosan and saturn on nitrogenas activity (µ mol C_2H_4 /g/dry wt./hr^{-1}) of *Azolla pinnata* . The values listed are the means of 3 replicated ± SD (stander Deviation)

Treatment / Period (days)	Control	furadan				hinosan				saturn			
	Concentration (ppm)												
	0.00	0.001	0.002	0.003	0.004	0.001	0.002	0.003	0.004	0.001	0.002	0.003	0.004
0	**5. 13**	**5.13**	**5. 13**	**5. 13**	**5.13**	**5.13**	**5.13**	**5. 13**	**5.13**	**5.13**	**5. 13**	**5.13**	**5.13**
5	**19. 50** ± 0.51	**14.15** ± 0.93	**18. 05** ± 0.24	**16. 00** ± 0.50	**15. 22** ± 0.38	**11. 84** ± 0.17	**10. 57** ± 0.18	**13. 00** ± 0.17	**7. 14** ± 0.26	**10. 51** ± 0.87	**12. 34** ± 0.38	**8. 14** ± 0.76	**7. 04** ± **0.21**
10	**12. 51** ± 0.59	**11.71** ± 0.15	**21.78** ± 0.23	**17. 22** ± 0.34	**11. 17** ± 0.25	**12. 13** ± 0.17	**13.78** ± 0.08	**12. 11** ± 0.26	**6. 23** ± 0.29	**5. 22** ±0.32	**6. 24** ± 0.57	**7. 17** ± 0.31	**5. 00** ± 0.26
15	**22.14** ± 0.92	**13. 50** ± 0.33	**15.75** ± 0.14	**14.13** **± 0.12**	**14. 10** ± 0.23	**7. 15** ± 0.40	**8. 25** ± 0.10	**7. 52** ± 0.35	**8. 00** ± 0.17	**7.18** ± 0.45	**9. 52** ± 0.52	**5. 23** ± 0.08	**5. 47** ± 0.40
20	**9. 41** ± 0.66	**11. 66** 0.09	**22. 50** ± 0.28	**11. 05** ± 0.17	**6. 78** ± 0.33	**7.75** ± 0.18	**11. 35** ± 0.05	**8. 18** ± 0.20	**5.14** ± 0.20	**4. 35** ± 0.17	**7. 42** ± 0.33	**4. 60** ± 0.42	**3. 79** ± 0.33
25	**17.24** ± 0.69	**7.74** ± 0.10	**19. 60** ± 0.11	**6. 50** ± 0.15	**7. 13** ± 0.25	**6. 50** ±0.53	**7. 82** ± 0.08	**6. 55** ± 0.22	**3. 33** ± 0.13	**6. 40** ± 0.55	**4. 51** ± 0.10	**3. 35** ± 0.13	**4. 22** ± 0.28
L.S.D.at 0.05 P Conc.xtime		0.6092				0.7370				0.5651			

Doubling time

The results obtained (Table 3) showed that the doubling time at lower concentrations (0.001, 0.002 ppm) of furadan and hinosan were significantly decreased than the control, especially after 20 and 25 days. However, the herbicide saturn has an inhibitory effect on *Azolla* doubling time even at low concentration (0.001ppm) since it showed a significant increase in doubling time, as compared with the control and the other two pesticides. Singh et al. (1988) and Madhaiyan et al. (2006) suggested that the application of various insecticides and fungicides showed low toxicity affects on the doubling time of *Azolla*, compared with herbicides. Xiaofeng et al. (2008) added that *Azolla* doubling time would be clearly shortened when grown in artificial controlled environmental condition and so its biomass increased.

Dinitrogenase activity

The data represented in Table (4) showed that dinitrogenase activity of *Azolla* treated with the tested pesticides gradually increased with time, till 10 or 15 days incubation, then an inconsistent decrease was obtained after 20 and 25 days.

Moreover, maximum dinitrogenase activity was generally obtained at 0.002 ppm furadan, throughout the incubation periods. These values were higher than those of hinosan and saturn at different concentrations and also than the control. A similar trend was reported by Holst et al. (1982) who found that the insecticide carbofuran (furadan) significantly increased dinitrogenase activity of *A. pinnata*. Moreover, it was increased by application of a low concentration of lindan (Singh et al., 1984). On the other hand, Ismail et al. (1995) found that the herbicide saturn reduced the growth and dinitrogenase activity of *A. pinnata*. On the other hand, Madhaiyan et al. (2006) reported that addition of pesticides to the growth media substantially reduced the dinitrogenase activity of pure cultures of *G. Diazotrophicus.*

NPK uptake

The data in Figures (1), (2), (3) showed that nitrogen, phosphorus and potassium uptake was generally increased with increasing the incubation period of the applied furadan and hinosan, nearly at all concentrations. The highest NPK uptake by *A. pinnata* was obtained with the medium concentration (0.002 ppm) of both pesticides after 20 and 25 days of

incubation. *Azolla* fronds grown in a medium supplemented by saturn at different levels showed inconsistent decrease in NPK content with lapse of time. It was obvious that NPK values at different saturn concentrations, were significantly lower than those of the control and the other two pesticides (furadan and hinosan). These results agreed with those of Arrora et al. (2003), who reported that NPK, calcium and magnesium are very important and produce marked effects on the fern growth. It was found that dry weight and total nitrogen had a higher concentration with P (Madhaiyan et al., 2006), nitrogen (Wettern, 1985 and Hechler and Dawson, 1995) and K supply (Liu, 1987). It was obvious from the results obtained throughout this experiment that *A. pinnata* showed higher tolerance to furadan and hinosan at the different concentration used, as compared with the lower concentrations of saturn.

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An Analysis of Polyethylene Coating Corrosion in Oil and Gas Pipelines

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Abstract: The corrosion of pipelines' coatings is one of the main problems in oil and gas industries for which a large amount of money is spent each year. Coating is the first defense line in front of a corrosive environment in which pipes have been buried. Good function of coating depends on its adhesiveness rate to the metal surface. Initial adhesiveness and its durability in the contact conditions are among those factors that enhance coating efficiency in long term. The rate of Initial adhesiveness has a high relationship with coating movement and surface wetness by this movement in the course of applying the coating and also with cleanliness and preparedness of pipe surface. The durability and permanence of adhesiveness depends on coating properties including its resistance in front of moisture penetration. Applying coating on the pipelines has a high cost so for this reason the selection and application of coating is of high importance. Also for underground buried pipes it is not possible to change their coatings in short durations unlike other structures. Therefore the coating must be durable for 20 years. This article proceeds to investigate the reason for corrosion in steel pipes with three poly ethylene layers.
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Keywords: corrosion ; initial adhesiveness ; poly ethylene coating.

1. Introduction

The corrosion of pipelines' coatings is one of the main problems in oil and gas industries for which a large amount of money is spent each year. Cessation of production creates a very high loss in terms of hydrocarbon production or maintanence costs. Therefore equipment faultless during their shelf life is considered as a basic problem .Those studies which result in compilation of effective strategies, laws, protocols and methods for preventing and removing corrosion effects are studied as ;corrosion management;. Corrosion problem in Canada has resulted in ten times pipelines' leakage and twelve times explosions in the period of 1977 to 1996, and in our country investigating this phenomenon and its management is of extraordinary higher importance due to the fact that oil, gas and petrochemical industries have been located in corrosive environments.

The reports of malfunctions due to corrosion indicates that the reason for this phenomenon is mainly due to tragic carelessness in plumbing and equipment manufacture and installation which result in explosion, fire and spread of toxic materials in living environment. besides it has some costs such as replacement of corroded equipment, shut down of plants due to replacement of corroded equipment, disturbance in processes due to equipment corrosion and impurity of processing products due to corrosion –related leakage and waste of the products of those vessels which are attacked by corrosion, all of these problems make the most important costs and losses created by corrosion. The studies show that 70 percent of losses can be prevented by observing related principles and instructions. According to the report of Bartel institute one third of industries 'corrosion costs are prevented by simple applying of existing knowledge and technology. Another point which is ignored is that indirect corrosion damages are much more than direct ones.Corrosion management has the responsibility of corrosion control and installations in all respects for preserving capital and always uses advanced tools and methods in enhancing this purpose.

Corrosion process is managed since the very beginning of planning installation until their servicing by corrosion manegement.For example a planning engineer gathers enough information from corrosion management to design structures with long and useful shelf life or amends the following work steps by using enhanced information from occurred corrosions.

2. Under Coating Corrosion Mechanisms

Under Coating Corrosion is started in presence of water and oxygen. When water and oxygen are present on the surface of a metal, corrosion occurs due to metal dissolution (anodic effect). This chemical process is balanced by oxygen reduction. Under Coating Corrosion rate depends on the kind of insulation, the amount of oxygen, the amount of impurities in the water, temperatures and the heat transfer properties of metal surface or the conditions of metal surface being wet or dry. In the abscence of oxygen the amount of corrosion rate can be ignored .Although low alloy and carbon steels

have the lowest corrosion rate in alkali environments but chloride ions create localized pitting under coating. If sulphur and nitrogen acids having acidic properties penetrate in to insulation through impurities of water and air or if water has acidic property, general corrosion occurs. Sometimes impurities of water and air specialy nitrate ion (NO3) cause external stress corrosion cracking (SCC) in unstrained carbon or low alloy steels. The phenomenon is more pronounced when intermittent wetting and drying of environment cause the development of impurity concentration.

2.1. Corrosion Control Methods

Corrosion in industries is controlled by one of the following methods.

A-Corrosion-resistant alloys
B- Corrosion inhibitors
C-Stabilization method
D- Corrosion-resistant alloys

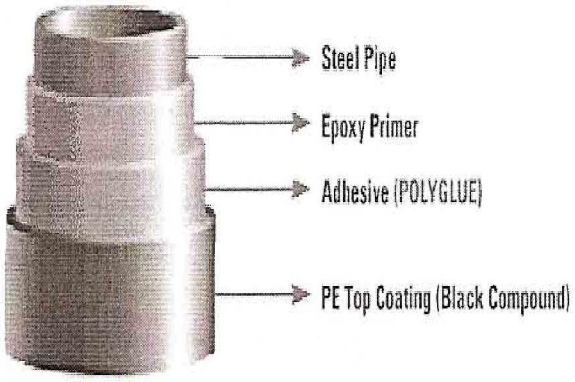

Figure 1: A view of steel pipe with three fold cover.

3. Coatings and their roles in country's economy and industry.

It is quite clear that any of the coating systems have their own adventages and limitations, and that is why one of them is preferred over the other in most of the conditions. But in most other conditions both two systems can be used and it makes selection difficult. In these occasions there must be a suitable method for investigation and comparison that is a reliable guide in selecting proper system. One of the important factors in selecting proper system is cost. The importance of cost factor is such that it is dominant over other parameters and cause selection of a system based on cost. The coating of pipelines exposes a lot of items during operation such as moisture, pressure, bacteria and etc…..

Applying coating over pipelines has a lot of costs, for this reason selection of coating is of much importance.

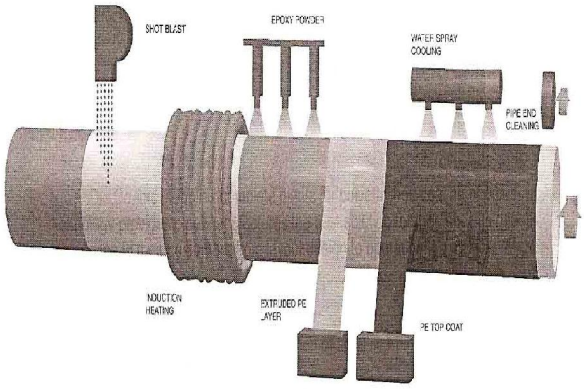

Figure2: View of cover process

Also for buried pipes underground there is the possibility that their coating must be replaced in short durations like other structures and the coating must last at least for more than 20 years. For this purpose the properties a coating needs is as follows:

1-Resistant against water and moisture: even dry soils have a little moisture and pipeline coating is often wet, for this reason coating mustn't absorb moisture because it results in weight increase and electrical resistant reduction.

2- Resistant against variable pressures: placement of pipes under ground results in pipes being under pressure. Also the presence of gravel, movement of soil due to moisture and also other existing particles in the soil causes the above mentioned variable and unharmonious pressure. In fact coating must be a physical protection and not separate from surface.

3- Resistant against bacteria and mushrooms: There are a lot of bacteria in the soil which attack different materials and cause their extinction. Of course bacteria and mould attack is not so prevalent.

4- Resistant against water capillary effect: Water penetration due to capillary effect causes separation of coating from steel. Any fine crevice or gap causes the capillary effect unless the contact between coating and pipe is strong and very sticky. In fact primer color has the duty of creating a strong adhesiveness between pipe and coating and prevents water penetration and coating separation.

5-Suitable with temperature variations: Temperature variations can be influential because the rate of steel expansion and coating is different. Expansion and shrinkage result in movement in the pipe but this movement is uniform and slow. For this reason coating must be resistant against temperature variations and not separated from the pipe.

6- Resistant against being solved: Water is capable of solving some of the materials but the

coatings are insolvable in water. Also it must be investigated that coating be resistant against other solvents besides being insolvable in water specialy against oil and its derivatives.

7- Resistant against absorbing soil: Soil may absorb some materials. Clay, silica gel, charcoal and some other combinations have the absorbing property. Soil always is completely in the contact with coating and absorption of some elements from coating by the soil may make coating fragile, perforated or reduce its resistance against soil.

8- Resistant against mechanical damages: besides the aforesaid items in part 2, coating must be resistant to mechanical stresses during installation or storage.

3.1. First layer

Immediately after the pipe one form of film of liquid or gum of epoxy is created. Minimum dryer thickness must be between 20-60 micron. Based on ISO 2808, epoxy powder has some materials which are used against heat that is used for three-layered poly ethylene coatings for steel pipes and must be specially formulated and designed and this is for electrical application and corrosion improvement from coating system and also providing unlimited cathode maximum resistance is suitable. Epoxy powders used in three –layered coatings is classified in two different groups. The first group has primer property and the second group has coating quality.

These two materials have remarkable differences in applying, temperature and thickness; there is a tendency in industries to use epoxies with coating quality .Epoxy layer must have such an enough thickness that prevent holiday formation. priences and experiments done in the field indicates 40 holiday in 40 feet for a layer with the thick ness of 150 micron.

According to Dennis Neal, the manager of Harding and Neal Company of USA having experience in coatings and corrosion recommends minimum thickness of 250 micron for the epoxy layer. Time is a sensitive and critical factor in creating adhesive and poly ethylene layers. First the adhesive develops a very strong chemical bond with chemical groups in epoxy powder which is uncure therefore into this stage the epoxy must not completely cured. On the other hand adhesive and poly ethylene are connected physically which is done by rollers' pressure and time being critical and sensitive is because of epoxy for bond with adhesive must not completely cure on one hand and must get jelly condition on the other to be able to resist against rollers' pressure, in the other words all operations of these steps are done in less than a second.

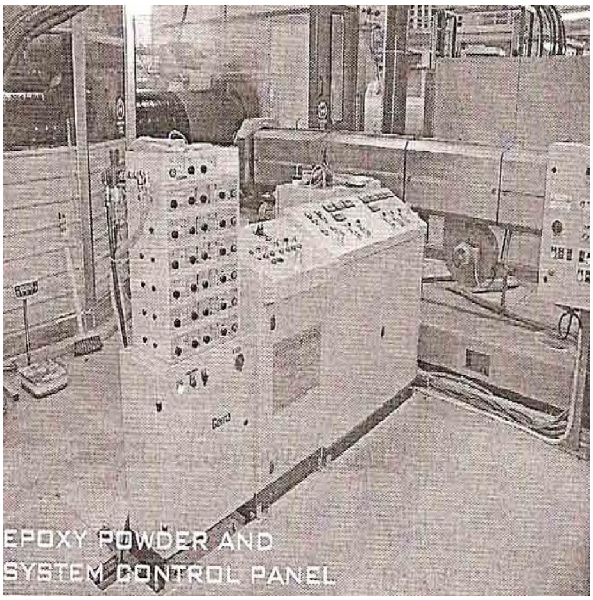

Figure 3: view of epoxy controller.

Coating appliers must be careful that applying a solution for three-layered coating dosent result in another problem for example separation in the seams is reduced by lowering applying temperature of epoxy from 239.4 'C to less than 232.2'C, but although FBE is cured in lower temperature, high viscosity of molten in this temperature dose not allow epoxy flow and complete wetting of metal surface and this causes adverse effect on coating adhesiveness in warm condition and moisture anh extra catholic voltage . The following is Dennis Neal's opinion about this matter obtained from experts:

i) The FBE layer is under cured because the application temperature is low to allow the adhesive to chemically bond to FBE.

ii) There is no adhesion between the FBE and adhesive because the temperature is higher and the FBE is fully cured before the adhesive is applied.

3.2. Second layer

Second layer polymer creates adhesiveness between layers 1 and 3 and must be compatible with both layers. Minimum thickness must be between 160-200 micron. Thickness may increase or reduce according to the mutual agreement with customer but minimum thickness must be investigated safely.

Table 1: Physical properties of adhesive.

PROPERTY	UNIT	VALUE
1)Density	g/cm^3	0.900-0.950
2)Melting index (2.16 kg/190˚C)	g/10min	0.5-8 or as suitable for application as PE (top coat)
3)Elongation	%	95 (min)
4)Melting point	˚C	
5)Co monomer content	%	9 (Typical)

Note: The test for raw epoxy power properties is under the responsibility of manufacturer.

3-3-Third layer

Polyethylene coating must be formed in this layer. Thickness must be uniform in all through the pipe and minimum general thickness must be acceptable.

4. Conclusion

Generally a lot of national capital is spent for corrosion costs in the country. By a short glance on other countries' experiences it is observed that most of the countries have invented ways to prevent corrosion-related damages and applied them.

Corrosion experts in America have felt that there is a basic need for conducting studies to estimate metal corrosion costs incured by America's economy and to prepare a strategy to reduce corrosion-related costs. In this vein according to the dialogues between National America Corrosion Engineers (NACE) and members of transportation ministry, a plan was submitted for the cost of corrosion in 21 century which was accepted in 1998.In 2001 corrosion cost project was submitted in America that in this report direct corrosion cost was estimated by analysis of 26 sections of industry which had a complete information about corrosion.

Finally the total direct cost of corrosion in America was estimated to be 276.000.000.000 dollars in a year. Also an indirect cost of corrosion was equal to direct costs. Most of the experts in the country believe that we need abasic movement in preparing complete formal statistics about corrosion at first so that the dimensions of corrosion are specified in all industries. In the next step we can force industries to consider a series of least corrosion in their management by preparing a preventive strategy for controlling corrosion with the help of assembly. Preventive strategy may be as follows:

1-Developing the awareness about the high cost of corrosion and potentials of cost reduction

2-Changing this wrong attitude that nothing can be done for reducing corrosion costs.

3-Changing policies, rules, standards and management excercies to reduce corrosion costs by corrosion effective management.

4-Enhancing instruction and skills of employees to identify corrosion control methods.

5-Reviewing the procees of designing products to prevent corrosion costs increase.

4.1. Strategies for corrosion management

Corrosion management proceeds to offer preventative strategies in two technical and no technical domains. The topics of no technical domain as preventative strategies are as follows:

1-Enhancing the employees' awareness about the high costs of corrosion and saving costs result in correct applying of existing technologies and corrosion costs. Thus a lot of corrosion problems are due to lack of awareness about corrosion management and accountability of people in exchanging operations, inspection and maintenance of management system.

2- Changing guidelines, protocols, standards and management methods to reduce corrosion costs by correct corrosion management resulting in effective control of corrosion and safe operation and increase in shelf life of equipment.

3-Amending and generalization of employees' instruction to introduce and identifying corrosion control.

4-Changing and amending wrong belief about not being able to do anything about corrosion and making new decisions in preventing this phenomenon. Also preventive strategies in technical domains are of a very high importance. Some of these strategies are as follows.

A-Upgrading planning methods and using advanced planning ones to better managing corrosion which prevents avoidable corrosion costs.Inthis vein planning methods must change and the best corrosion technologies must be available for planners.

B-Improving corrosion technologies via research and development. Corrosion can be controlled in most industries by using scientific methods and new technological achievements.

4.2. An analysis of reasons for three-layered poly ethylene coating separation.

Good function of coating depends to a high extent to its adhesiveness rate to metal surface. Initial adhesiveness and its durability in contact condition are of those factors that result in high efficiency of coating in long term. The extent of initial adhesiveness has a very high relationship with coating flow and its wetting when applying coating

and also with cleanliness of surface and its readiness. Durability of adhesive depends on coating properties such as its resistance against moisture penetration and also its endurance against cathodic disbandment.

The most leading coatings having more consumption than other kinds are as follows:

1-FBE (fusion bonded epoxy)

2-Poly urethane (from technical view poly urethane materials are of the best coatings used since 1970 on).High cost of this coating has resulted in using it just for special cases such as when temperature is very high. Three-layered poly ethylene coating includes epoxy, adhesive and poly ethylene.

Any of the layers provides coating with properties to lengthen its efficiency for a long term. Epoxy layer has a very good adhesiveness due to its transverse bonds and has a very high resistance against corrosion and oxygen penetration. But it is vulnerable to the mechanical hit when storing and line performance. Poly ethylene layer is a very good protection to prevent physical damages. A main problem with this coating is that poly ethylene does not have adhesiveness with the metal and for this reason an adhesive layer being a kind of reduced polymer is used for pasting poly ethylene to epoxy.

4.3. Main factors in coating separation are as follows:

1-The manner three-layered poly ethylene coating (quality) of applying coating in the factory

2-ExposurConditions and properties

Three-layered poly ethylene is one of these coatings with high efficiency, although it seems that it is used in the field in a very limited extent (comparing other coatings) and more laboratory studies and field experiences are needed to investigate if they have aforesaid properties.

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Scrutiny Water Penetration in Three-layer Polyethylene Coverage

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Abstract: Coverage in line pipes include of high costs. For this selecting cover and how apply is high important. Three fold polyethylenes include of epoxy layers, adhesive and polyethylene. Each other from layers having attributes that increasing its application for long term. Polyethylene layer is good shelter for prevent of physical damages. In attention to corrosion in lower temperature is a electrochemical reaction and rate of a electrochemical reaction is very impress of a element or very reactor from surface. This position occurred when influence of a element increasing of other cover controllers. A example of this issue that will be cause of outer corrosion in pipes under soil and this is very importance in work, this is leakage water into covers that can be measurable with coefficient of water leakage that can exchanging layers quality. This article has studied leakage water into three fold polyethylene cover.
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Keywords: leakage water; polyethylene cover; epoxy layer; outer corrosion.

1. Introduction

Pipe coverage technology improved contemporary with spanned of gas and oil liens. Today for cover using from synthesis rosin and in industries installing over pipes with high attention processes.

Cover is first defense line against corrosive background that pipes are under it. Second defense line is cathodic custody that is vital element for the pipes remained set. In the other hand custody current is taken corrosion cover then for sureance from custody we would increase efficiently of cover with good installing cover in metal surface in pipes.

Efficiently of cover very depend on its stick to pipe surface. Primitive scale of sticky depend on applying cover, wetting surface by it when covering, also clear and ready of pipe surfaces. Strength and survival in stick depend on cover properties, such as: strength against leakage wet also for cathodic disbandment.

2. Properties of cover

Properties that a cover need:

Strength with water and wet; even dry soil have a percent of wet and coverage line pipes. In most cases is wet, then for this cause cover shouldn't attracted wet, for because attraction. Water will be cause of increasing weight and also decrease electrical strength. Resistance to variable pressures; putting under soil lead to pressure on pipes. Also existence of soil particle of wet and other exist. Particle in soil is cause of creation this variable and uncoordinated pressure. In fact cover should be one physical cover and don't abruption from surface. Resistance to bacteria, fungus: there are many bacteria in the soil that attacked to varied material and are caused of destroyed of materials. Of course attacked by fungus or molds not prevalent.

Resistance for water's capillary: water's influence because of capillary is a cause of analyze cover from steel and all cracks and tiny whole are this effect unless contact between cover and pipe are strength and very sticky. In fact primer color has such duty that product very sticky and cover, that preventing of water pervade and dissenting cover.

Prepare with temperature's cost: costing of temperature can be affecting because coal of steel's expansion and cover are difference. But usually this work is slowly and steady for this reason cover should have strength to charging temperature and with this work, don't release of pipes. Resistance with solution. Water is able to solved into some material but usually coverage unsolved to water and also it should be study that this is unsolvable. And it is resistance to other solvent particularly against of oil and its derivations. Resistance to attractive soil: soil may be attracted some material. Clay, silica gel, wood coal and other something have solvent nature, soil is quietly connecting to cover and attracting of some elements from cover by soil may do, brittle porosity soil or decreasing strength to corrosion.

3. Cover process

3.1. First layer:

Immediately after pipe a form create by a film from liquid or rosin from epoxy powder. Minimum of diameter (thick) of 4444 should be 20-60 micron (by ISO2808) [2].

3.2. Epoxy layer

Epoxy powders that are useable to three fold covers, divide two groups; first group that have primer nature and second group have cover's quality. This two units have noticeable differs in setup position and temperature and thickness.

Generally in industries, tendency for use of epoxies with cover quality. Epoxy layer should have been enough thickness that finally will have better properties for system.

Study mother list of UK Company Jotun Powder Coating showing that epoxy layer are over150 micron thickness particular into greatest pipes Epoxy layer should have been enough thickness until avoiding from reveal of holiday. First experiments and test that setup in this place, revealing and increasing over 40 holding in 40 foot for level 150 micron. With attention to recommendation's Dennis Neal, master of Hording and Neal U.S.A Company that have a long history in coverage say that minimum of thickness for epoxy layer should be 250 micron.

Time for implementing adhesive layers and polyethylene on epoxy are a momentous and critical. First, adhesive establishing firm consociation chemical with chemically groups that still are uncured, but for this step epoxy shouldn't raised quietly. In other hand adhesive and polyethylene connect together physically, this work doing with pressure of roller and being sensible to time is for this reason that from one hand epoxy for connect to adhesive shouldn't be cure and other hand should be such gel until can be resistance to pressure of roller or all of these stages should be setup less than some second. Exalters of cover should be look for that use of a solution to three fold cover problems wouldn't be cause of existence of other problem.

For example, separate in frontier with decreasing temperature epoxy from 239.4 ˚C decrease to less than 232.2 ˚C however FBE release in lesser temperature don't allow to current of epoxy and wetting quietly. Surface's metal and this work have inverse effect to sticky cover for heat and wet situation and voltage will more than cathodic [1].

3.3. Second layer

Producing sticks between layer 1&3 and it should be compatible with each two layers. Min of thickness would be between 160-200 micron. Thick may be differ in its rage (decrease or increase) with agreement to client but min thickness should be study, safely

3.4. Third layer

Polyethylene cover would form in this level. Thickness would be steady in overall pipe and general min of thickness would be acceptable [4].

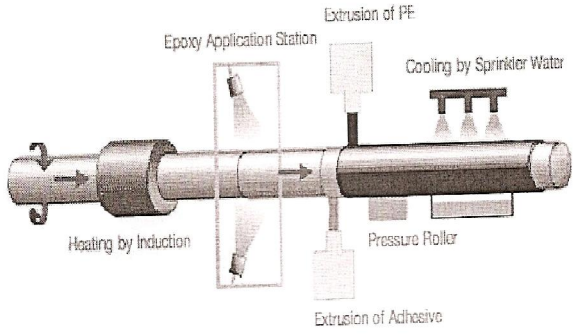
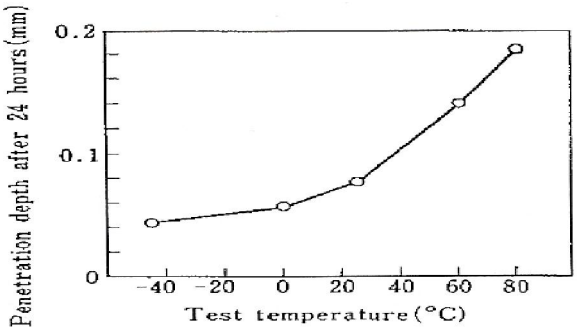


Figure 1: general view of covering

4. Leaking water test

Leakage test (style: DIN 30670) had done in basic of heavy particle test from coverage polyethylene pipes (three fold cover). Relation between leak of water after 24 hours and temperature is shown this follow [6].

Chart 1: Relation between temperature and leakage test after 24 hours.



Have seeing that with increasing temperature, also leakage increased.

5. Conclusion

Setup quality is engineer's way for notice to however owner of line wanted in shape of consumer. Outer covers pipes are good guidance of increasing quality for constructors. Under the corrosion begin in the present of water and oxygen. When water and oxygen are in surface of metal, occur corrosion of metal's dissolution (Anode effect).

This chemical process balanced with decrease oxygen. Under the corrosion scale depend on cover type, oxygen scale that available, and impurity scale into water, temperature and properties of transferring temperature for surface metal and dry and wet position. In absence of oxygen corrosion scale is consumer regardless.

Although carbon steels and low alloyed usually in alkaline environments have scale of corrosion, chloride ions (Cl^-) are cause of local vesicular corrosion (localized pitting) under the cover: If sulfur acid and nitrogen, that have acid property, inter to insulator with impurities in water and air or if water have been acid property, sometime occurred general corrosion. Impurities of water and air, particularly nitrate ion ($NO3^-$) can be cause of tension fraction.

(SCC) for out and under covering in steel carbon or alloyed low that didn't de tension. current phenomenon, particularly when intermittent dry and wetting process of environment.

5.1. Cover effect

Corrosion occurred under each of covers. Types of this depend on rate and quality only. Most of cover effect in this type corrosion, doing safety convoluted place for aggregation and survive of water.

Water can aggregated from outer sources of rain or liquids of condense. Chemical syntax and also cover attributes have a role in corrosion. Cover can attracted water and have a good background for chemical reactions indeed chloride and sulfate into cover can acting like electrolyte and increasing corrosion surfaces.

5.2. Temperature effects

Temperature in surface of metal has a two fold role in corrosion cover. Controlling corrosion under very heat covers is difficulty from other cool cover. Because of vaporized water under cover and increasing density of impurities follow water. In closed systems high temperature, speeding up chemical reactions but in open systems high temperature, increasing corrosion also high temperature decreasing age of preservation covers.

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Effect of Feeding Different Sources of Energy on Performance of Goats Fed Saltbush in Sinai

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Abstract: Feeding halophytes is a feasible solution to minimize the problem of feed shortage in arid and semiarid areas of Egypt. This work aimed to investigate the effect of feeding goats on fresh *Atriplex nummularia* which is grown naturally and cultivated in Sinai on performance of growing goats when added with different sources of energy supplementation (concentrate feed mixture CFM, ground barley grains or ground date stones and mixture of these materials) on nutrients digestibility, nitrogen balance, water utilization and some rumen and blood metabolites. The experiment was performed on twenty eight of growing goats (six months old) with mean body weight 16 ± 0.38 Kg were divided into four equal groups for 105 days. The diets were given at the basis of 60% concentrat for growth requirements , roughages were offered as ad-lib. The roughages were berseem hay in T1 (control group) or fresh *Atriplex nummularia* in T2, T3 and T4 whereas the energy supplements were concentrate feed mixture (CFM) in T1, ground date stones in T2, ground barley grains in T3 and a mixture of 50% ground barley grains with 50% ground date stones in T4. Results obtained revealed that inclusion of barley grains in T3 group improved DMI of Atriplex than that in T1, T2 and T4 groups. The highest body weight gain was recorded by animals in T1 and T3 compared to those of the other treatments. In addition Intakes of TDN and DCP were maximum in T1 and T3. The maximum apparent digestion coefficients of OM, CP, EE and NFE were recorded by animals in T3 while those of DM and CF were digested much better by animals in T1. TDN% and DCP% were increased in T1 followed by T4. All animals were in positive nitrogen balance. The maximum values of total water intakes were recorded for animals in T2 whereas the lowest values for animals in T3 with significant differences. Serum creatinine, total protein, globulin and AST levels were not affected by diet type and they were within the normal ranges. Also a sampling time factor was detected. Ruminal ammonia- nitrogen and total volatile fatty acids revealed significant variations before feeding and 6 hrs post feeding. The feed cost of daily gain (L.E)/ kg was achieved for animals fed ground date stone in T2 (L.E 0.860) which was lower than T4, T3 and T1 (L.E. 1.255, 1.273 and 1.290) respectively. In conclusion, barley grains or ground date stones or their mixture improved the nutrients utilization and intake of *Atriplex.* Utilization of such halophytic plants supplemented with non–conventional energy supplements could be recommended to enhance feed materials availability all-round year and to improve animal performance as well under arid and saline conditions of Sinai.

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Key words: Salinity, halophytes, saltbush, barley grains , date stone , goats, intake, nutrients digestion.

1. Introduction:

Agro-industrial by-products are available in Egypt in large quantities averaged (26 million ton) (El Shaer, 2004), some of such materials are characterized by high nutritive value (Youssef et al., 2006). So it can be used as supplementary feed ingredients in animal rations (Mohamed and El-Saidy, 2003). Date stone is one of this agro-industrial by- products used as feed ingredients under desert conditions which are available in abundancy all over the year as a main source of energy. It should be offered to ruminants in crushed or ground forms. Date stone have been demonstrated by many investigator as an acceptable, cheap and rich feed ingredients feedstuffs for sheep and goats (Shawket et al., (2001); Nasar, (2002) and Abdou, (2003).

Atriplex nummularia an ever green shrub, widely distributed and cultivated in Egypt along the Mediterranean Coastal Zone and the Suez Gulf (Shawket et al., 1998 and El-Shaer, 2006). *Atriplex* so called is known to be tolerant to drought and salinity (Ben Salem et al., 2002). It is high in crude protein, crude fiber and ash (sodium) but relatively low in carbohydrates (El-Shaer, 2004a and Ben salem et al., 2005). The previous authors reported that sheep fed on *Atriplex* alone decreased or at least maintained their live body weights. In the presence of energy sources like barley grains, *Atriplex* could proved to be a good, cheap source of nitrogen.

The aim of this study is to evaluate the effect of replacing barely grains by ground date stone as energy supplement sources for growing goats fed *Atriplex nummularia* as a basal diet in terms of

growth performance, economic, feed efficiency and some rumen and blood parameters.

Materials and Methods

The current work was carried out at Ras Suder Research Station, belongs to Desert Research Center, Southern Sinai Governorate. The experiment lasted for 105 days.

Experimental animals and rations:

Twenty eight growing black Desert goat kids Six months old with an initial live body weight of 16±0.38 kg were divided randomly into four equal groups (7 animals each) as follow:

T_1: Berseem hay (4th cut *Trifolium alexandrinum*) + concentrate feed mixture (CFM) as a control Treatment

T_2: *Atriplex nummularia* + Ground Date Stone (GDS).

T_3: *Atriplex nummularia* + Barley grains (BG).

T_4: *Atriplex nummularia* + (50% barley grains + 50% ground date stone).

Rations were formulated according to the recommended nutritional requirements for growing goats (Kearl, 1982). Diets were balanced on basis of 60% concentrates and fresh *Atriplex nummularia* was collected daily and left free choice for animals except T1 group which fed berseem hay.

A feeding trial followed by a digestibility trial was conducted and lasted for 105 days. Each 7-animal group was offered one of the above mentioned four treatments in a group feeding system. *Atriplex nummularia* was harvested daily and left free choice for animals. During the feeding trial, animals were weighted every two weeks and nutrients requirements were adjusted according to the change in their live body weight. Feed offered and refused were daily weighed to calculate dry matter intake (DMI). Drinking water was available for all animals during the whole feeding trial.

Digestibility trial:

Four animals from each treatment were randomly selected for the digestibility trail, at the end of the feeding trial, as a 15-days adaptation period followed by 5 days collection period. All the animals were kept in separate individual metabolic cages and 90% of their feeding requirements were offered to each animal where accurate records were kept for feed and water intakes and animal excreta during the collection period. Measured amounts of drinking water was available for each animal daily , then daily water intake was calculated and recorded , the composite samples of feed offered and feces were dried, ground and kept for further chemical analysis by the end of collection period. A common method of assessing metabolizable energy intake (MEI) is use of body weight change or gain as an indirect measure (Luo et al., 2004).

Rumen liquor and blood sample:

During the last three days of collection period, rumen liquor was sampled before feeding, and 6 hours post feeding by stomach tube. Simultaneously, blood samples were taken from the jugular vein before feeding and 6 hours post feeding in dry clean centrifuge tube, left to clot for 30 minutes at 37°C and then centrifuged at 3000 r.p.m. for 10 minutes. Serum was separated, divided into five aliquots and stored at -40°C to be thawed only once on demand.

Chemical analysis:

Proximate analysis for feed, feces and urinary nitrogen were analyzed according to A.O.A.C. (1997). Natural Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent lignin (ADL) were determined according to Goering and Van Soest (1972).

Rumen total volatile fatty acids (TVFA's) were tested (warner, 1964) and ammonia nitrogen values were also evaluated (A.O.A.C. 1997). Blood serum samples were assayed for total protein (Armstrong and Carr1964), albumin (Doumas and Biggs 1971). Globulin was obtained by substracting the total proteins values from the albumin values. Serum creatinine (Henry1965) and urea (Patton and Crouch1977). Aspartate amino Transferase (AST) and Alanine amino Transferase (ALT) (Schmidt and Schmidt 1963) were tested. All blood serum analysis were measured using Jenway spectrophotometer (UK) and using kits purchased from Human Company (Germany).

Statistical analysis:

General linear model procedure was used for statistical analysis using **S**AS (1998). The used design was one way analysis. Duncan's multiple tests (1955) were applied for means comparison.

3. Results and Discussion

Chemical composition:

Chemical composition for barley, ground date stone, Concentrate Feed Mixture as energy feed supplements and *Atriplex nummularia* and berseem hay as basal diets are displayed in Table (1). The results showed that DM, OM, EE, CF and cellulose contents are higher in berseem hay than that of *Atriplex nummularia*. Fresh *Atriplex nummularia* and berseem hay had comparable values of CP. Similar results were obtained by Abdul-Aziz et al, (1999). On the other hand, fresh *Atriplex nummularia* contained

higher ash, NFE, NDF, ADL and Hemicellulose than that of berseem hay. Such findings are acceptable since *Atriplex nummularia* is halophytic saltbush which is rich in ash fiber constituents. The results are in good agreement with many investigators tested the saltbush as animal feed materials (Le Houerou, 1994,Shawket et al, 2001and El -Shaer, 2006).

Data of Table (1) showed that DM and OM were higher in barley and date stone than that of concentrate feed mixture (CFM). However, CP content of CFM was higher than that of barley or date stone respectively. Barley grains contained lower ash and higher NFE than that of date stone and CFM which were comparable. However, date stone contained higher CF, EE, NDF, ADF, ADL, cellulose and Hemicellulose than that of barely grains.

Metabolisable energy concentrations (ME kcal/kg DM) of individual feedstuffs in this study differed markedly from each other. Barley grains was the richest supplement (2.88 kcal/ kg DM) followed by Date stones, CFM, Atriplex then BH, respectively (2.74, 2.16, 1.74 then 1.73 Mkcal/kg DM), respectively. Metabolisable energy pattern could be attributed to TDN % of each feedstuff.

Table (1): Chemical composition and feed ingredients of diets fed to goats (as DM basis %)

Items	Hay	Atriplex	barley	Date stone	concentrate feed mixture CFM
DM	85.321	36.68	95.60	94.32	89.66
OM	85.86	76.30	96.60	96.50	95.44
Ash	14.14	23.70	3.40	4.50	4.56
CP	12.75	12.25	9.03	8.15	14.2
CF	28.65	17.08	6.38	13.67	8.14
EE	1.69	1.59	2.1	4.88	3.36
NFE	42.77	45.38	79.09	68.80	69.74
Fiber constituents %					
NDF	58	60.30	18.00	71.90	52.75
ADF	43.15	42.81	7.00	56.08	9.78
ADL	13.45	17.89	2.00	12.99	4.65
Cellulose	29.70	24.92	5.00	43.09	5.13
Hemicellulose	14.85	17.49	11.00	15.82	42.97
ME, Mcal/kg DM	1.73	1.74	2.88	2.74	2.16

T_1: Berseem hay + concentrate feed mixture (CFM) as a control treatment
T_2: *Atriplex nummularia* + Ground Date Stone (GDS). T_3: *Atriplex nummularia* + Barley grains (BG).
T_4: *Atriplex nummularia* + (50% barley grains + 50% ground date stone).
ME,M cal/ kg DM = (TDN × 3.6) /100 (Church and Pond, 1982).
a, b, c and d : values with different letters in the same row means statistically significant at $P<0.05$

Body weight changes and intakes**:**

Average values of body weight changes and feed intakes are illustrated in Table (2). Response of animals fed different rations was varied and hence, the resulted final live body weights among treatments were varied. Body weight changes for all experimental animals were generally positive and all kids increase in weight. Animals of the control group (T1) revealed the highest percent body weight change all over the experimental period.

Average daily gain of T1 did not differ significantly from that of T4 & T3. Animals fed on *Atriplex* plus 100% ground date stones (T2) had the lowest ($P \leq 0.05$) gain averaged 34.7 g/ day.

Body weight changes, kg % of initial weights were 33.64, 32.61, 29.33 and 21.89 for T1, T4, T3 and T2 in descending order. Such findings were recorded earlier using Black Bedouin kids by El-Shaer et al. (1996) and El-Hassanein et al., (2002).

Similar results were obtained by Shawket et al. (2001 & 2002) who found that replacing 50% of barley grains by 50% date seed led to appreciation increase of ADG.

Total dry matter intake (TDMI) approximately did not differ significantly among treatments. They recorded comparable values of TDMI. Similar trends were observed by Shawket et al. (2001). The differences in dry matter intake of basal diet (gm/kg B.W) were not differ significantly among the experimental treatments. It was high in T3 which may be attributed to mutual associative effect between *Atriplex* and barely grains. The results are in harmony with the findings of (Kandil and El-Shaer (1990) and El-Shaer et al., 2001 & 2002) who found that the utilization of barley grains as energy supplement in sheep diet resulted in an increase in feed intake of roughage.

There are non significant differences in intake of energy supplement among treatments. Total intakes of basal diets and feed supplements revealed insignificant differences. It was noticed that maximum intake was record in (T3) and the minimum was in (T4). Such reduction in dry matter intake in (T4) might be due to date stones content of ADL compared with barley grains and CFM. This result was similar to that of El-Shaer et al, (2002) and El-Hassanein et al., (2002). In general, the present results indicated that intakes were considerably affected by the type of diet. Moreover, higher fiber constituents mainly ADL and NDF were known to reduce DM intake (El-Shaer, 1995). Changing the type of energy supplement by replacing barley grains instead of date stone not significantly increased nutrient digestibilities.

It is noted that the types of energy supplements did not affect the digested nutrients intakes (TDN) but significantly ($P \leq 0.05$) influenced the digested crude protein (DCP,g/Kg BW). Growing kids fed the control consumed the highest amounts of DCP (3.25g/Kg BW), while the lowest one (1.79g/Kg BW) was recorded for animals in T2. The goat fed the rations containing Atriplex plus barley grains (T3) consumed amounts of digested nutrient (TDN) nearly equal those of the control ration (T1). These findings revealed that *Atriplex* and berseem hay showed similar digestibilities. The results revealed, also, that the average daily gain for successive diets appeared to be affected by TDN and DCP intakes where feeding the rations of T1 and T3 to the experimental animals resulted in higher daily gain compared to the other groups. These results proved that addition of barley grains improve the total digestible nutrients (TDN) intake which resulted in increment in daily gain. These findings could be related to higher CF content of date stones which decreased significantly the digestibilities of other nutrients (Shawket et al., 2002). This may reduce dietary readily available carbohydrates resulted in reduction of the nutrients digestibilities of diet.

It is concluded that growing goats fed on unconventional feeds (Atrilpex, barely grains or ground date stones) appeared to consume and utilize the TDN and DCP similarly to those fed conventional feed (CFM + berseem hay). Such results are in agreement with those reported by Abdou (1998), Abdul Aziz et al. (1999), El-Shaer et al., (2001 & 2002) and Shawket et al. (2001).

Table (2): Body weight changes, average daily gain and intake of goats fed the experimental diets.

Items	T_1	T_2	T_3	T_4	± SE
No. of animals	7	7	7	7	
Initial L.B.W, Kg	16.14	16.64	16.57	16.16	
Final L.B.W, Kg	21.57	20.28	21.43	21.43	1.27
Body weight changes	5.43	3.64	4.86	5.27	1.49
% of initial weights	33.64	21.89	29.33	32.61	
Average daily gain, gm /day	51.71^a	34.70^b	50.90^a	50.19^a	0.56
DM intake gm/kg B.w.					
Supplement	24.89	22.41	22.50	21.35	0.82
Roughage (basal diet)	14.85	15.96	18.24	16.54	1.06
Total DM intake	38.94	38.37	40.74	37.89	1.16
Digested nutrient intake g/kg B.W.					
TDN	22.67	21.37	22.05	21.42	0.82
DCP	3.25^a	1.79^c	2.20^b	2.12^b	0.10
MEI (kcal / kg $BW^{0.75}$	140.4	131.1	140	139.6	2.32

T_1 : Berseem hay + concentrate feed mixture (CFM) as a control treatment
T_2 : *Atriplex nummularia* + Ground Date Stone (GDS). T_3 : *Atriplex nummularia* + Barley grains (BG).
T_4 : *Atriplex nummularia* + (50% barley grains + 50% ground date stone).
a,b,c Means with different superscripts in the same raw are significantly different at ($P \leq 0.05$)
MEI = 457+(25.23XADG) ($g/kg^{0.75}$) (Luo et al., 2004)

.Digestion coefficients and nutritive value:

Data of Digestion coefficients and nutritive values (Table 3) showed that DM, OM, CP, EE digestibility did not differ significantly by changing the types of energy supplements as animals tended to digest such nutrients at comparable trends. However, animals fed *Atrilpex* plus barley grains (T3) exhibited greater values of OM, CP and EE digestibilities than those recorded for goats in the other groups; the lowest values was recorded for animals of T2. It may be due to the low contents of ADL, ADF and NDF contents of barley grains compared to other feed supplements. Furthermore, replacing barley grains with ground date stones in T2 resulted in decreasing

digestibilities of DM, OM and CP. Similar trends were reported earlier on small ruminants fed saltbush supplemented with non-conventional energy supplements (Hassan and Abd El-Aziz, 1979, Abou El- Nor et al.., 1995 and El- Shaer et al. 1996). The apparent digestion of crude fiber as well as all fiber fractions were varied significantly (P≤0.05) among treatments. It was noticed that inclusion of ground date stone alone (T2) and barley grains (T4) with *Atriplex* improved digestion coefficient of NDF, ADF, and cellulose (T4) and ADL and hemicellulose (as in T2). Indeed, it might be due to higher cellulolytic bacteria in the rumen of animals fed on date stones (Kandil and El-Shaer, 1988).

Nutritive values expressed as TDN% and DCP% revealed significant (P≤0.05) differences among groups. The addition of barely grains as a sole energy supplement (T3) or mixed with ground date stone (T4) to *Atriplex nummularia,* as a basal diet, improved the nutritive values of the rations. Such results are in close to those reported by El-Shaer et al. (1996), El-Shaer et al. (2001) and Shawket et al. (2002). Significant increment of TDN and DCP in T1 might be attributed to concentrate mixture and higher digestiblities of nutrients in this treatment. It is clear that TDN % was affected by energy source in diet. These results are in agreement with those reported by Etman and Soliman (1999).

Metabolizable energy intake (MEI) data for growing goats were comparable with slight differences among them. These differences were a reflection to average daily gain (ADG) values. Animals of control group (T1) showed the greatest MEI (140.4 kcal/kg BW $^{0.75}$) followed by animals fed T3 (140.0 kcal/kg BW $^{0.75}$) followed by animals fed T4 and at last animals fed T2 (139.6 and 131.1 kcal/kg BW $^{0.75}$), respectively. These differences might be partially attributable to two reasons: 1) differences in metabolic activity of tissues because of different energy supplements and 2) experimental conditions where the studied animals were kept under normal farm conditions hence, greater energy use for activity would be expected (McDonald et al., 1977). MEI estimates for growing goats were greater than that (103.01 kcal/kg BW $^{0.75}$) determined by Luo et al.(2004). This difference might be attributable to experimental conditions. In publications used by Luo et al. (2004) most goats were housed in relatively small areas, such as metabolism chambers or crates, whereas goats in the publications assed in the present study were kept under normal farm conditions hence greater energy use for activity would be expected. Similar findings and explanations were recorded by Luo et al. (2004a).

Table (3): Digestion coefficients, nutritive value of goats fed the exprimental diets.

Items	T_1	T_2	T_3	T_4	± SE
Digestion coefficients %					
DM	75.51	70.18	71.68	72.93	2.47
OM	77.76	69.47	79.04	76.73	2.91
CP	75.29	70.51	76.00	74.96	2.11
CF	63.57^{a}	62.83^{a}	45.77^{b}	60.58^{a}	3.14
EE	79.72	73.96	80.83	77.90	2.56
NFE	74.15^{ab}	70.63^{b}	82.61^{a}	82.18^{a}	2.51
NDF	63.75^{a}	65.77^{a}	51.35^{b}	67.31^{a}	3.97
ADF	47.91^{c}	63.68^{ab}	51.90^{bc}	66.68^{a}	3.85
ADL	39.80^{ab}	52.27^{a}	32.51^{b}	46.33^{ab}	4.85
Cellulose	52.90^{c}	68.32^{ab}	63.68^{bc}	76.2^{a}	3.61
Hemicellulose	74.89^{a}	78.08^{a}	62.48^{b}	68.96^{ab}	3.8
Nutritive value					
TDN %	78.40^{a}	69.80^{b}	75.50^{ab}	76.76^{a}	2.50
DCP %	9.80^{a}	6.89^{b}	7.52^{b}	7.66^{b}	0.25

T_1: Berseem hay + concentrate feed mixture (CFM) as a control treatment
T_2: *Atriplex nummularia* + Ground Date Stone (GDS). T_3: *Atriplex nummularia* + Barley grains (BG).
T_4: *Atriplex nummularia* + (50% barley grains + 50% ground date stone).
a,b,c Means with different superscripts in the same raw are significantly different at (P ≤ 0.05).

Nitrogen utilization and water intake:

Data in Table (4) revealed that nitrogen utilization (mg/kg BW) in terms of nitrogen intake (NI), nitrogen excretion and retention were affected significantly (P≤0.05) by the types of energy supplements among the four treatments. However, Nitrogen intake of kids fed saltbush supplemented with ground date stone or / and barley grains did not

vary significantly between T2, T3 and T4 since the animals consumed relatively similar amounts of nitrogen (10.49,10.29 and 10.54 mg/kg BW, respectively). NI of animals in T1(control) was significantly (P≤0.05) higher than that fed the experimental treatments (T2, T3, T4); it might be attributed to high CP content of CFM than barley and date stone(Table 1). Total nitrogen excretion followed the same patterns of NI and fecal nitrogen excretion Where animals fed saltbush supplemented with different energy sources excreted slightly similar(P≥0.05) amounts of N through feces and urine. The total N excretion was highest (11.48 mg N/Kg BW) for animals fed the control ration in T1.

Concerning nitrogen retention, there were significant (p≤0.05) variations among treatments where all sheep were in positive nitrogen balance and retained significant various amounts of nitrogen. The highest NB was recorded in T1. Utilization of ground date stone in T2 instead of barley grains (T3) led to a decrease in nitrogen balance by about 46.15. It seems that the pattern of daily body weight gain of the treated animal groups was matching with the pattern of nitrogen retention where animals retained more nitrogen tended to gain higher body weight and the opposite was true ((P≤0.05) Table 2). The results are in harmony with those obtained by Allam et al., (1997) and El- Shaer et al., (2001).

Data of Table (4) showed that drinking water and feed water intake varied (P≤0.05) significantly among treatments. Goats in T2 consumed higher amount of drinking water while those fed T4 showed the lowest amount of drinking water. The highest value of feed water intake was recorded for animal in T4 while the lowest value was recorded in T1 due to low moisture content of berseem hay fed to animals of T1. This reduction in free water intakes for experimental diet groups, compared with control group was attributed mainly to the high moisture content of *Atriplex* compared with berseem hay. It is, also, appeared that animals in T4 were able to digest NDF, ADF and cellulose (Table3) better than their mates in other treatments due to the lowest drinking water consumption (Table 4). These results are in agreement with several investigators (Abou El-Nasr, 1985; El -Shaer et al. 2002 and Shawket et al .2002).

Table (4): Nitrogen utilization and water intake of goat fed the experimental diets.

Items	T_1	T_2	T_3	T_4	± SE
Nitrogen utilization:					
Nitrogen intake (NI) (mg/kg Bw)	15.80^{a}	10.49^{b}	10.29^{b}	10.54^{b}	0.86
Fecal nitrogen (FN) (mg N/kg Bw)	4.64^{a}	3.15^{b}	3.05^{b}	4.01^{b}	0.36
urinary nitrogen (Un) (mg N/kg Bw)	6.84^{a}	5.38^{ab}	3.60^{b}	3.45^{b}	0.76
Total nitrogen excretion (TNE) (mg N/kg Bw)	11.48^{a}	8.53^{b}	6.65^{b}	7.46^{b}	0.79
Nitrogen balance (NB) (mg N/kg Bw)	4.32^{a}	1.96^{b}	3.64^{ab}	3.08^{ab}	0.38
NB % of intake	27.34	18.68	35.37	29.22	3.49
Water intake ml/kg BW					
Drinking water. (Free water)	157.26^{a}	136.44^{ab}	116.07^{c}	99.46^{bc}	8.30
Feed water	8.69^{b}	39.67^{a}	18.60^{a}	40.86^{a}	3.002
Total water intake	165.95^{ab}	176.11^{a}	134.67^{b}	140.32^{ab}	10.28

T_1: Berseem hay + concentrate feed mixture (CFM) as a control treatment
T_2: *Atriplex nummularia* + Ground Date Stone (GDS). T_3: *Atriplex nummularia* + Barley grains (BG).
T_4: *Atriplex nummularia* + (50% barley grains + 50% ground date stone).
a,b,c Means with different superscripts in the same raw are significantly different at (P ≤ 0.05).

Rumen liquor characteristics:

There were significant (p≤0.05) differences in ammonia-N concentrations and total VFA's (as illustrated in Table 5) among experimental animals before and 6 h post feeding which could be related to the significant changes of TDN and DCP of the tested diets. Punia and Sharma (1990) reported that total VFA's concentration and its production were higher for barley and molasses as a source of energy. Similar results were reported by Hatfield et al.(1998) and Abou'l Ella et al. (2005) who found that total VFA's and ammonia –N concentrations were significantly (P≤0.05) increased with further increases in the nutritive values of the diet.

The NH3- N concentration was affected by time where it was the minimum before feeding and increased significantly (P≤0.05) 6hrs post feeding in all treatments. Similar trends were observed by Ibrahim et al. (2001).

Table (5): Rumen characteristics of goats fed the experimental diets.

Criteria	Sample time	T_1	T_2	T_3	T_4	Mean
Ammonia- nitrogen	0	31.618^{cb}±0.94	12.76^{d}±1.32	26.28^{c}±3.30	26.69^{c}±0.85	24.34^{b}±1.99
NH3-N	6	33.89^{b}±0.83	14.77^{d}±1.93	41.04^{a}±4.03	41.38^{a}±1.31	32.77^{a}±2.99
Mean		32.75^{a}±0.72	13.76^{b}±1.15	33.66±3.69	34.03^{a}±2.87	
Total volatile fatty acids	0	3.91^{b}±0.163	2.87^{c}±0.177	5.44^{a}±0.35	5.50^{a}±0.293	4.43±0.31
TVFA's	6	5.17^{a}±0.123	4.0^{b}±0.591	3.98^{b}±0.355	3.95^{b}±0.13	4.27±0.21
Mean		4.54^{a}±0.26	3.44^{b}±0.36	4.71^{a}±0.38	4.73^{a}±0.33	

T_1: Berseem hay + concentrate feed mixture (CFM) as a control treatment
T_2: *Atriplex nummularia* + Ground Date Stone (GDS). T_3: *Atriplex nummularia* + Barley grains (BG).
T_4: *Atriplex nummularia* + (50% barley grains + 50% ground date stone).
a,b,c Means with different superscripts in the same raw are significantly different at (P ≤ 0.05)

Blood parameters:

Table (6) summarizes the data of blood biochemical parameters of the studied experimental animals. Blood urea levels revealed significant variations (P≤0.05) among tested diets at both 0 and 6 h post feeding. The highest level of urea was found in animals fed the Berseem hay (T1) where BH was the richest N diet in the present study followed by animals fed on T3 as a reflection of DCP pattern among the treatments could be attributed to the presence of tannins in *Atriplex nummularia* which form a complex compound with protein and adversely affect N digestion in rumen (Reed et al. 1990, Romero et al., 2000 and El-Shaer et al. 2005). There was no significant difference in urea levels in fasting and 6 hrs post feeding when neglecting diet type.

Creatinine level was neither affected by sample time nor by type of diet indicating normal renal function. Similar results were obtained by Shawket et al. (2001).

Blood proteins profiles showed that the total protein (TP) concentration was not significantly different among the tested groups, but it revealed significant differences (P≤0.05) when time factor was included. The mean value of TP in the fasting state was higher than that recorded in 6 hrs post feeding.

Serum albumin and globulin concentrations showed the same trend when the types of the tested diet were ignored (P≤0.05). Hoffman et al. (2001) reported that animals fed on high protein diets had significantly higher total protein, albumin and total globulins than those on low protein, Total protein concentration of serum increased in response to the rising level of rumen concentration of ammonia (Abou'l Ella et al., 2005). Although nitrogen intake was differed significantly among groups but blood proteins not follow the same trends.

Albumin levels were affected significantly with the type of diet (P ≤0.05) whereas levels of globulin were not. Animals fed T2 had the least concentrations of albumin. It is known that change in albumin level reflect the change in liver function because the liver is the sit of albumin synthesis.

Serum Aspartate amino transferase (SAST) enzyme level was not differ with the time of sample but it was slightly affected by the diet. Serum Alanine amino transferase (SALT) values mentioned non significant variations among groups. It is clear that AST and ALT concentrations were in normal ranges although animals fed on tanniferous rations as previously reported indicating that the experimental animals were in good health and showed no hepatotoxicity and these results coincided with those of Romero et al. (2000).

Data of Table (7) found that the animal fed the control diets was more feed efficiency. expressed as kg DM/ kg gain (12.16) while the lowest was T2 (18.4). TDN kg/kg gain was higher in T2 (9.82) while T1, T3 and T4 had comparable value. These finding may be attained by increasing the level content of CF, NDF, ADF and ADL in date stone than that of CFM and barley. Similar results were obtained by Allam et al., (1997) and Abdul- Aziz et al. (1999), The best utilization efficiency of DCP was found with animals fed *Atriplex* + unconventional energy source (barely or date stone) . Data of table (7) showed that the economical efficiency was affected by type of roughages and concentrate. Data indicated that kids fed T2 and T4 were more economic efficiency for production one kilogram gain of body weight followed by T3 (12.67LE/ kg gain). These results indicated that concentrate which contain date stone

had minimum price for production one kilogram by about 54, 48.89% than that of control (T1) which fed on CFM and berseem hay and by about 41.99, 34.41% than that (T3) which fed on barley grains plus *Atriplex*

Table (6): Blood metabolites changes of goats fed the experimental diets.

Criteria	Sample time	T_1	T_2	T_3	T_4	Mean
urea	0	38.31^{ab}±0.867	25.31^{c}±1.064	41.42±5.36^{a}	33.94^{a}±1.75	34.75±2.032
mg/dL	6	40.79^{a}±1.064	28.72^{c}±1.74	34.05^{ab}±8.07	26.79^{b}±3.05	32.59±2.429
Mean		39.55^{a}±0.789	27.02^{c}±1.14	37.73^{ab}±4.69	30.37^{b}±2.11	
creatinine	0	0.616^{b}±0.050	0.902^{a}±0.152	0.948^{a}±0.064	0.716^{a}±0.0363	0.795±0.054
mg/dL	6	0.863^{ab}±0.085	0.639^{b}±0.081	0.809^{a}±0.029	0.773^{ab}±0.046	0.759±0.034
Mean		0.739 ±0.059	0.77 ±0.094	0.878 ±0.042	0.744 ±0.034	
Protein profile						
Total protein	0	8.13±0.188	8.36±0.585	8.19±0.184	7.89 ±0.255	8.14^{a}±0.160
mg/dL	6	7.72^{a}±0.125	6.13^{a}±0.613	7.57^{a}±0.494	7.31^{a}±0.402	7.18^{b}±0.257
Mean		7.925^{a}±0.129	7.24^{a}±0.576	7.88±0.271	7.61^{a}±0.257	
Albumin	0	3.92^{ab}±0.176	3.55^{b}±0.164	4.05^{ab}±0.177	4.15^{a}±0.096	3.92^{a} ±0.091
gm/dL	6	3.57^{ab}±0.182	2.97^{c}±0.253	3.84^{ab}±0.089	3.74^{ab}±0.228	3.53^{b}±0.124
Mean		3.74^{a}±0.134	3.26^{b}±0.177	3.49^{a}±0.99	3.94^{a}±0.124	
Globulin	0	4.21^{ab}±0.228	4.82^{a}±0.596	4.22^{ab}±0.121	3.75^{ab}±0.280	4.26^{a}±0.186
gm/dL	6	4.16^{ab}±0.162	3.16^{b}±0.402	3.71^{ab}±0.417	3.59^{b}±0.398	3.65^{b}±0.185
Mean		4.18 ±0.130	3.99±0.456	3.97±0.221	3.67 ±0.185	
Liver Enzymes						
AST	0	27.83^{b}±2.58	22.0^{c}±3.37	18.55^{c}±1.614	18.875^{c}±2.21	21.81^{a}±2.26
U/L	6	35.33±3.46	17.38^{c}±1.375	17.0^{c}±1.23	17.38^{c}±2.03	21.77^{a}±3.19
Mean		31.58^{a}±2.17	19.69^{b}±1.56	17.78^{b}±1.33	18.13^{b}±2.27	
ALT	0	4.00^{c}±0.03	4.00^{c}±0.01	4.10^{c}±0.10	4.40^{c}±0.40	4.13^{b}±0.135
U/L	6	5.33^{ab}±0.18	4.60^{bc}±0.38	5.60^{a}±0.33	4.50^{c} ±0.25	5.01^{a}±0.179
Mean		4.66±0.266	4.30 ±0.210	4.85 ±0.32	4.45±0.179	

T_1 : Berseem hay + concentrate feed mixture (CFM) as a control treatment
T_2 : *Atriplex nummularia* + Ground Date Stone (GDS).
T_3 : *Atriplex nummularia* + Barley grains (BG).
T_4 : *Atriplex nummularia* + (50% barley grains + 50% ground date stone).
a,b,c Means with different superscripts in the same raw are significantly different at ($P \leq 0.05$)

Table (7): Feed and economical evaluation of goats fed the experimental diets.

Items	T_1	T_2	T_3	T_4
Price of feed intake head/ day LE*				
concentrate	0.591	0.237	0.624	0.399
Roughages	0.250	0.018	0.021	0.018
Total	0.841	0.255	0.645	0.417
Feed cost of daily gain L.E	1.290	0.860	1.273	1.255
Feed cost/ kg gain	16.26	7.35	12.67	8.31
Economical efficiency**	1.534	3.373	1.974	3.010
Feed efficiency (kg feed/ kg gain)				
DM	12.16	18.40	13.26	14.19
TDN	7.08	9.82	7.18	7.06
DCP	1.01	0.86	0.77	0.71

* Based on market price of (2008) (LE /ton). The price of ton on DM basis was as follows:
CFM 1450, barley 1600 and berseem hay, 850 L.E.
The price of 1 kg live body weight of goat: 25 L.E/ kg
** Economic feed efficiency is expressed as the ratio between the price of total live body weight gain and the price of feed consumed to that gain.
T_1: Berseem hay + concentrate feed mixture (CFM) as a control treatment
T_2: *Atriplex nummularia* + Ground Date Stone (GDS). T_3: *Atriplex nummularia* + Barley grains (BG).
T_4: *Atriplex nummularia* + (50% barley grains + 50% ground date stone).

4. Conclusion.

It could be concluded that T2 and T4 which contained 100%, 50% date stone respectively were highly recommended to be used as concentrate diet for goats. This may be due to the higher price of CFM and barley than the price of date stone. Also, berseem hay was expensive than the collected *Atriplex.*

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Factors Associated with the Distribution of the Invasive Bivalve Clams" *Donax Variabilis* (Say,1822)" at the Area of the Mediterranean Coast Preferred by Marine Fish Larvae, New Damietta, Egypt

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Abstract: New Damietta shore is one of the important areas for collection of the clams as well as mullet, seabass and seabream larvae which are reliable for marine aquaculture in Egypt. *Donax variabilis* was recorded for the first time in Egypt and because of its presence in the area of Damietta Maritime Port, larvae has come stuck with ships from the Atlantic Ocean where they were registered there. The density of *D.variabilis* increased in site I (718 / m^2) than in site II (415 / m^2). Water salinity (33.43 ± 4.59 mg/ L) in site I was less than the salinity of the sea, while it was almost similar to the salinity of the sea (36.94 ± 3.45 mg/ L) at site II. Nutrients concentration at site II were higher than that at site I, where it averaged 0.02 ± 0.01, 0.05 ± 0.03 and 0.26 ± 0.16 at site I and 0.05 ± 0.03, 0.34 ± 0.41 and 0.46 ± 0.36 mg/l at site II for NO_2, NO_3 and PO_4 respectively. Measured *Chlorophyll a* was high at site II (0.25 0.12 mg/m^3) compared to site I (0.25 0.12 mg/m^3), revealing the increase in phytoplankton biomass at site II. Crustaceans and molluscs were the most groups associated with clam's beds. *D.variabilis* cohorts appeared during summer months, this indicates that the population consists of only one spawning event. Length frequency of *D.variabilis* was essentially bimodal during the period of study. Three modes were recorded in June, 2008 at size classes of 7, 11and 20 mm of shell length.
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Key wards: Mediterranean coast- *Donax variabilis*- A biotic factors- Biotic factors

1. Introduction:

A bivalvia can acquire an important role in solving the problem of shortage and high price of animal protein in Egypt. Egyptian beaches has extended over long distances and it has become necessary to look at how the exploitation of bivalvia inhabiting these beaches. Bivalve molluscs are conspicuous members of sandy beaches (McLachlan *et al*., 1996).

The family Donacidae inhabits exposed intertidal sandy beaches and form worldwide, by far, the largest group living in such highly dynamic environments (Ansell, 1983; Brown & McLachlan, 1990). Since this group is present in the seaside which is unstable and have continuous environmental changes.

Biotic (food availability and intraspecific interactions) and a biotic (beach slope, swash energy, sand particle size, salinity, and chemical cues) factors play predominant roles in regulating alongshore distribution patterns (Defeo & de Alava, 1995; McLachlan, 1996; Gime´nez & Yanicelli, 2000 and Lastra *et al*., 2006). Also human activity may represent an additional regulatory agent, acting either directly, by removal of individuals, or indirectly, by removal or disturbance of potential competitors (Defeo and de Alava, 1995; Defeo, 1996a; Schoeman, 1996 and Brazeiro & Defeo, 1999).

Ecological preferences of *Donax* have been studied by Ansell & Lagardere, 1980 and Guillou & Bayed, 1991. McLachlan & Jamarillo (1995) have reviewed the zonation of organisms on sandy shores and have emphasized the strong control exerted on distribution by physical factors and the temporal variability of the component communities. As well as, the population dynamics of *Donax* species have been examined by Guillou, 1982; Maze & Laborda, 1988; Maze, 1990 and Tirado & Salas, 1998.

Clams are important recreational and commercial resources in many countries (McLachlan, et al., 1996). *Donax sp.* spreading in the northern coast of Egypt and be the favorite food among the population in coastal cities. Inshore waters of the Damietta region (North coast of Egypt) support nursery (larval or fry) areas for several commercially important species, especially

the common mullet, sea bream and sea bass (El-Ghobashy, 2009). The present study will be the link between its distribution and some important environmental factors. Also, it is focused on the influence of these factors on the same aspects of clam biology.

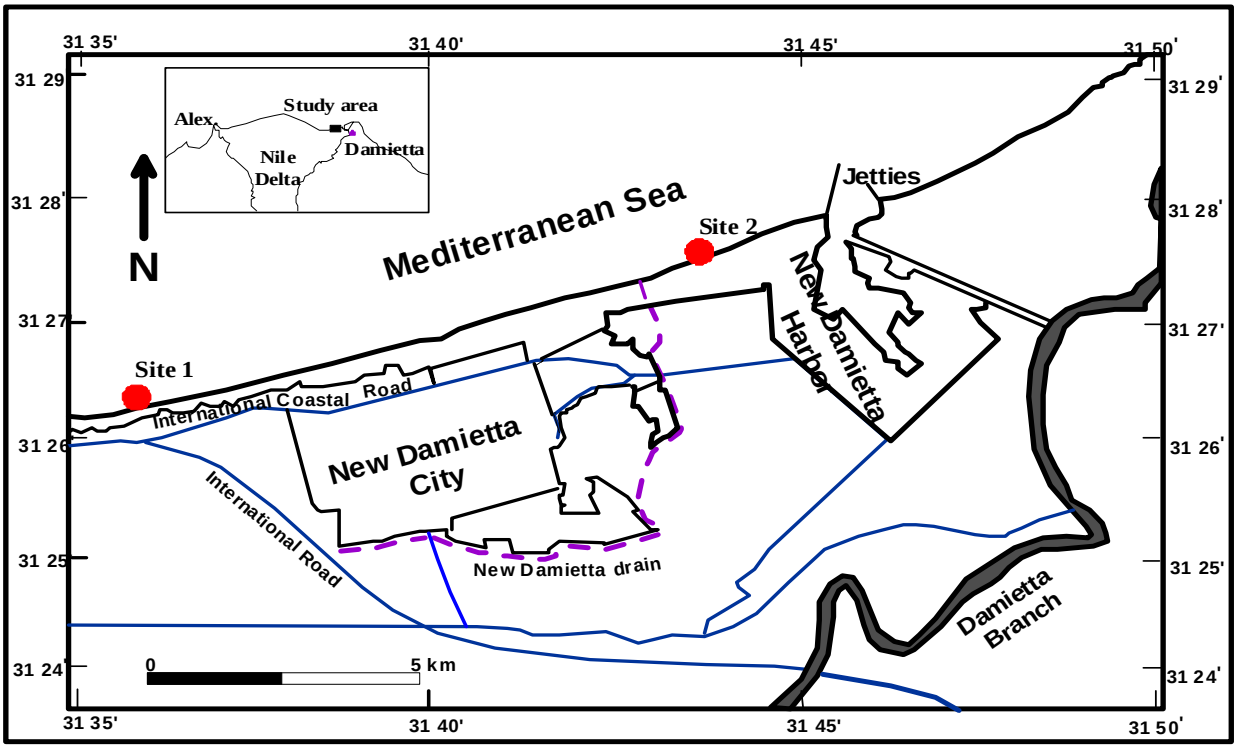
2. Materials and methods

Study area:-

The study area (Damietta shore) is an important part of the Egyptian coast of the Mediterranean Sea characterized by several important characteristics. i.e. fluctuation in the environmental qualities due to their proximity to the channel which reaches the Nile river with the sea as well as draw close to the Damietta Maritime Port. This segment is also the the most important location for collection of marine fish larvae upon which marine aquaculture activities taken place in the region. This area is also close to residential areas and activity of resorts and entertainment (Fig, 1).

Sampling sites:-

In order to determine the extent of *Donax* populations and also to select suitable sampling sites, a general survey of the New Damiettia shore using quadrates was undertaken in May, 2008. Consequently, two sites (Fig,1) were chosen, the first is located west of New Damietta, near the Gamasa drainage canal (31º 26.837 N and 31º 36.547 E) and the second is located east of New Damietta adjacent to Damietta Maritime Port (31º 28.577 N and 31º 44.408 E).



Fig, 1. Map of the sampling sites detected from land sat image.

Water Analysis:-

Monthly surface water samples were collected from the selected stations during the period from May 2008 to June 2009. A Plastic Ruttner water sampler of 2 liters capacity was used for water sampling. Water temperatures were measured using 0.1°C graduated Thermometer. At each station the total water depth and Secchi transparency was measured by the conventional Secchi Disc. The pH value was obtained using a portable pH-meter (Orion, model 6011). Dissolved oxygen (DO) was determined according to APHA (1985) using the Azide modification of Winkler method. Water salinity was directly measured using YSI model 339 (yellow springs) S-CT meter and the results are expressed as (g/L). Nitrate in the samples was determined according to APHA (1989) and Nitrite (Diazotization method) of samples was measured according to

Adams (1990). Total phosphorus in water sample was determined according to APHA (1989). Reactive silica in water sample was determined according to APHA (1989) and EPA (1983). The phytoplankton biomass (*Chlorophyll-a*) measured according to Strickland and Parsons (1972), using the SCOR UNESCO equations.

Sediment Analysis:-

The sediment samples were collected at the two studied sites by drilling to a depth of 50 cm. Sediment were screened with sieves of five grades, following the Wentworth scale: fine sand (125–250 µm); medium sand (250–500 µm); coarse sand (500–1000 µm); very coarse sand (1000–2000 µm); and gravel (>2000 µm). pH, TDS, EC, calcium carbonate were determined in soil extract according to APHA (1992). Organic carbon of the sediment samples was detected according to Adams (1990)

Collection and treatment of animals:-

Donax variabilis was collected in addition to other associated fauna from an area of one cupic meter. The collection was at the time of low tide using a specially designed hand dredge (75 cm wide) similar to that used by local fishermen but incorporating a smaller mesh size bag (3 mm) to survey the presence of smaller individuals which had not yet recruited to the professional fishery. Collected samples were kept in containers filled with 6% neutral formalin and it was brought to the laboratory at the Faculty of science, Damietta, where the investigations were carried out.

Statistics:

The comparison between means and standard errors was tested for significance using ANOVA analysis and Duncan's multiple range tests. In addition, the correlations of physicochemical parameters were assessed using Pearson's correlation analysis. All statistical analyses were calculated, using the computer program of SPSS Inc. (2001, version 11.0 for Windows) at the 0.05 level of significance.

3. Results:

Water salinity (33.43 ± 4.59 mg/ L) in site I was less than the salinity of the sea due to the impact of the Gamasa drainage canal, while it was almost similar to the salinity of the sea (36.94 ± 3.45 mg/ L) at site II. Nutrients (NO_2, NO_3 and PO_4) concentrations at site II were higher than that at site I. Their values were 0.02 ± 0.01, 0.05 ± 0.03 and 0.26 ± 0.16 at site I and 0.05 ± 0.03, 0.34 ± 0.41 and 0.46 ± 0.36 mg/l at site 2 for NO_2, NO_3 and PO_4 respectively. Measured *Chlorophyll a* was increased at site II (0.25 0.12 mg/m3) compared to site I(0.20 0.16 mg/m^3), this shows an increase in phytoplankton biomass at site II. Also electric conductivity (EC) increased in site II than that in site I reflecting the difference in the dissolved salts. It is also clear from the results; that there were very limited differences between the two sites for the rest of the factors were measured (Fig, 2).

Investigation of the sediment granules of the two studied sites indicated that, in site I the percentage of coarse and fine sediment were 1.82 and 3.50 with average diameter of 1.4 Mdφ, while in site II their raters were 1.80 and 3.22 respectively with mean diameter of 1.80 Mdφ. Scales of sediment in both sites were classified as medium sand. Mean percentage of organic content and pH in both sites were more or less similar in both sites. Site I had more bicarbonate, carbonate and organic matter (Table, 1).

Concerning with the abundance of associated fauna with clam bed, in the study area, it was clear that crustaceas and molluscs were the most dominated. Other groups such as annelids and echinodermates were rarely presented (Table, 2).

The density of D.variabilis per square meter in Site I ranged from 174 individuals during January to 1945 through July, revealing wide monthly changes in its density. In site II a narrow range (333-624 /m2) of fluctuations in D.variabilis were recorded. The fewest number of D.variabilis was during November (333/ m2) and December (322/ m2) while the highest number (624/ m2) was collected during March (Fig, 3). With regards to population structure, a shifting of the peaks from shorter to longer lengths can be observed from the length frequency histograms of D.variabilis (Fig, 4). Length frequency of D.variabilis at site I was essentially bimodal during the period of study. Three modes (including the juvenile one) were recorded in June 2008 at size classes of 7, 11and 20 mm of shell length. These modes represented 0.41, 4.10 and 17.21 % of the total population, respectively. Juvenile cohorts (3 to 7 mm shell length) were collected during the period from June 2008 to August 2008 and during July 2009. The largest length (25.12 mm) of D.variabilis was recorded during June 2009, which represented only 0.89 % of the total population. Growth curves of successive cohorts derived by plotting modal length classes as a function of time are shown in Fig, (6a.) Growth of the different cohorts proceeded with time and the modes of D.variabilis indicated a short life span of this species. D.variabilis cohorts appeared during summer months, this indicates that the population consists of only one spawning event. Monthly

average shell length increased of *D.variabilis* at New Damietta shore was 2.4 ± 1.2 mm.

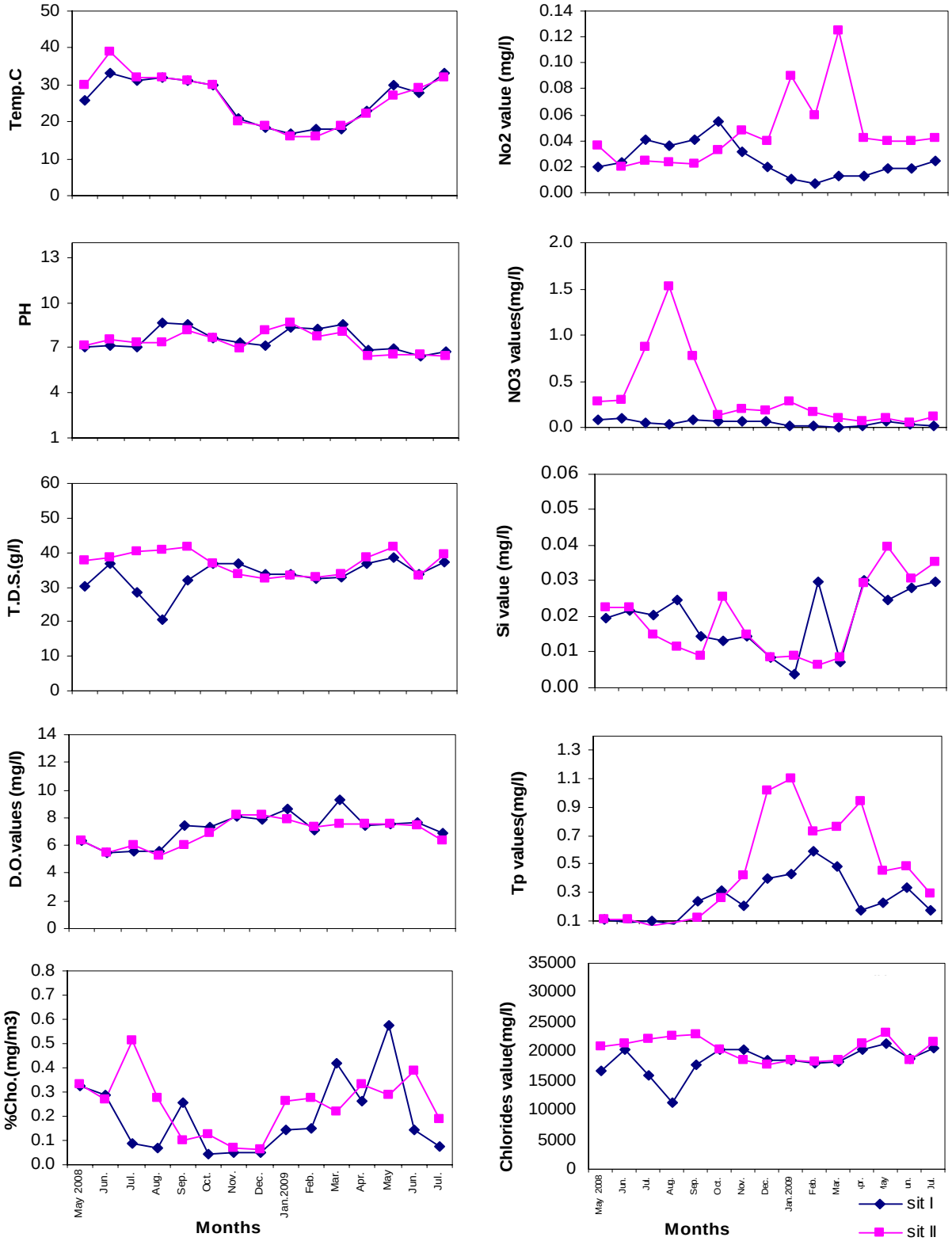
Length-frequency distributions for *D.variabilis* in the sit II (Fig, 5) were mostly bimodal. Three modals (including the juvenile one) appeared during April, August 2008 and July, 2009. Temporal appear of juveniles (< 6 mm shell length) was similar to that in site I. Their percentage occurrence relative to the whole population ranged from 14.84% in June, 2008 to 0.86% in July, 2009. Growth of the different cohorts proceeded with time and the modes of *D.variabilis* indicated also a short life span of this species. *D.variabilis* cohorts appeared during summer months, reflecting also one-spawning event. Monthly average shell length increased of *D.variabilis* at New Damietta shore was 2.4 ± 1.2 mm. The growth pattern of successive cohorts is shown in Fig, 6b. Cessation of growth was observed during winter months. The average growth rates of the different cohorts were 1.65 ± 0.5 mm/month. A highly significant difference ($P<0.002$) was calculated between *D.variabilis* growth rates at the two sampling sites.

Table (1): Sediment characteristics at the two sites in the New Damietta shore.

parameters	Site I	Site II
Coarse sediment >0.5 mm (%wt.)	1.82	1.80
Fine sediment <0.063 mm (%wt.)	3.50	3.22
Median diameter (Mdφ)	1.4	1.8
Wentworth scale	Medium sand	Medium sand
Quartile deviation (QDφ)	0.55	0.465
Categories of sorting	Moderately well sorted	Moderately well sorted
Quartile skewness ($Sk_q\varphi$)	0.05	0.04
PH	7.92	7.36
Chlorides	2958.33 mg/l	3481.07 mg/l
T.D.S	40.23 g/l	47.34 g/l
E C	2200 mmhos/cm	2800 mmhos/cm
Mean% Bicarbonate(Hco3) content	2.73	1.66
Mean% Calcium carbonate content	5.1	4.6
Mean% Organic carbon content	1.74	1.65
Mean% Organic matter content	3.51	3.32

Table (2): Monthly variations in the number occurrences of animal groups associated with *D.variabilis* at the two sampling sites.

Animal groups \ Months		May 2008	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan. 2009	Feb.	Mar	Apr.	May	Jun.	Jul.	Total
Annelida	Site 1				1					2			1				4
	Site 2				1			2			2						5
Arthropoda	Site 1	15	31	7	18	6	12	4	9	2	4	1	2	1			112
	Site 2	11	12	34	41	17	31	3	4	1	2	2	12	1	3	6	180
Mollusca	Site 1			1			10	29	19	12	4	6	3	30	5	2	121
	Site 2			5		7	6	12	25	20	10	15	29	41	16	19	205
Echinoderm ata	Site 1	1										1					2
	Site 2																0
Total	Site 1	16	31	8	19	6	22	33	28	16	8	8	6	31	5	2	239
	Site 2	11	12	39	42	24	37	17	29	21	14	17	41	42	19	25	390


Temp.C
No2 value (mg/l)
PH
NO3 values(mg/l)
T.D.S.(g/l)
Si value (mg/l)
D.O.values (mg/l)
Tp values(mg/l)
%Cho.(mg/m3)
Chlorides value(mg/l)
May 2008
Jun.
Jul.
Aug.
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sit I
sit II

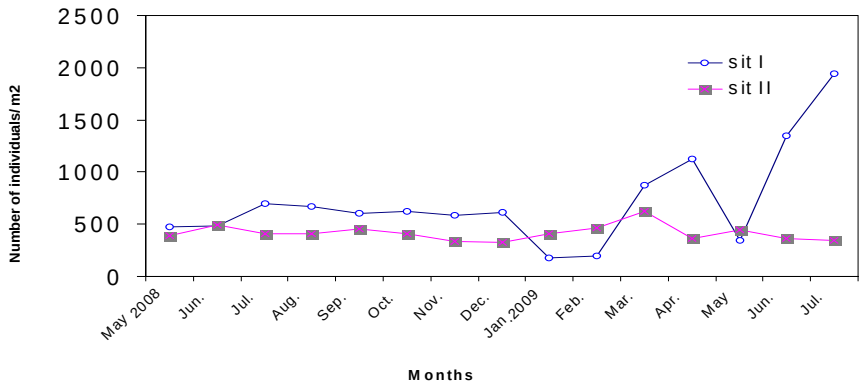


Fig. (3): The monthly change in the number of Donax variabilis /m² at two sites.

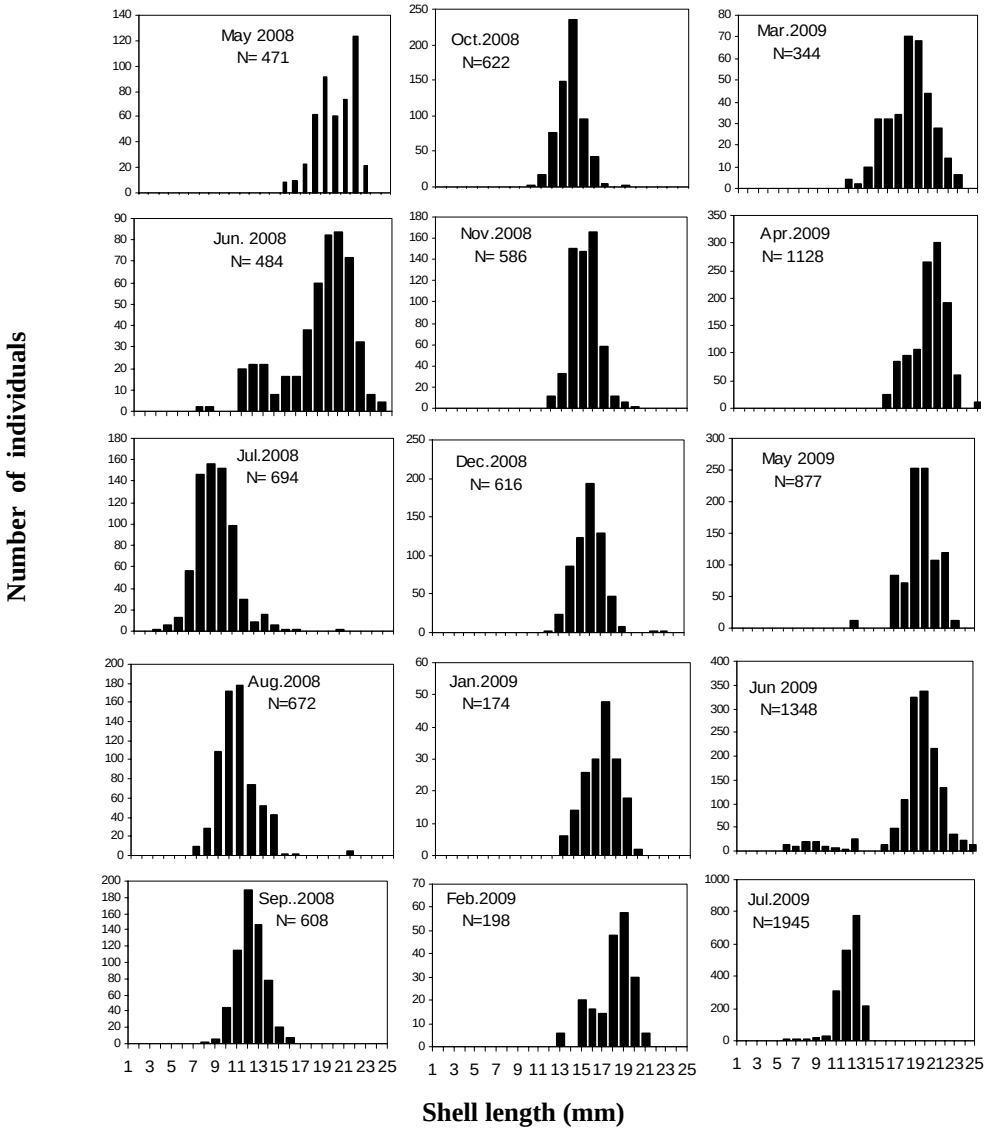


Fig. (4): Monthly size frequency histograms of *D.variabilis* samples collected from site I

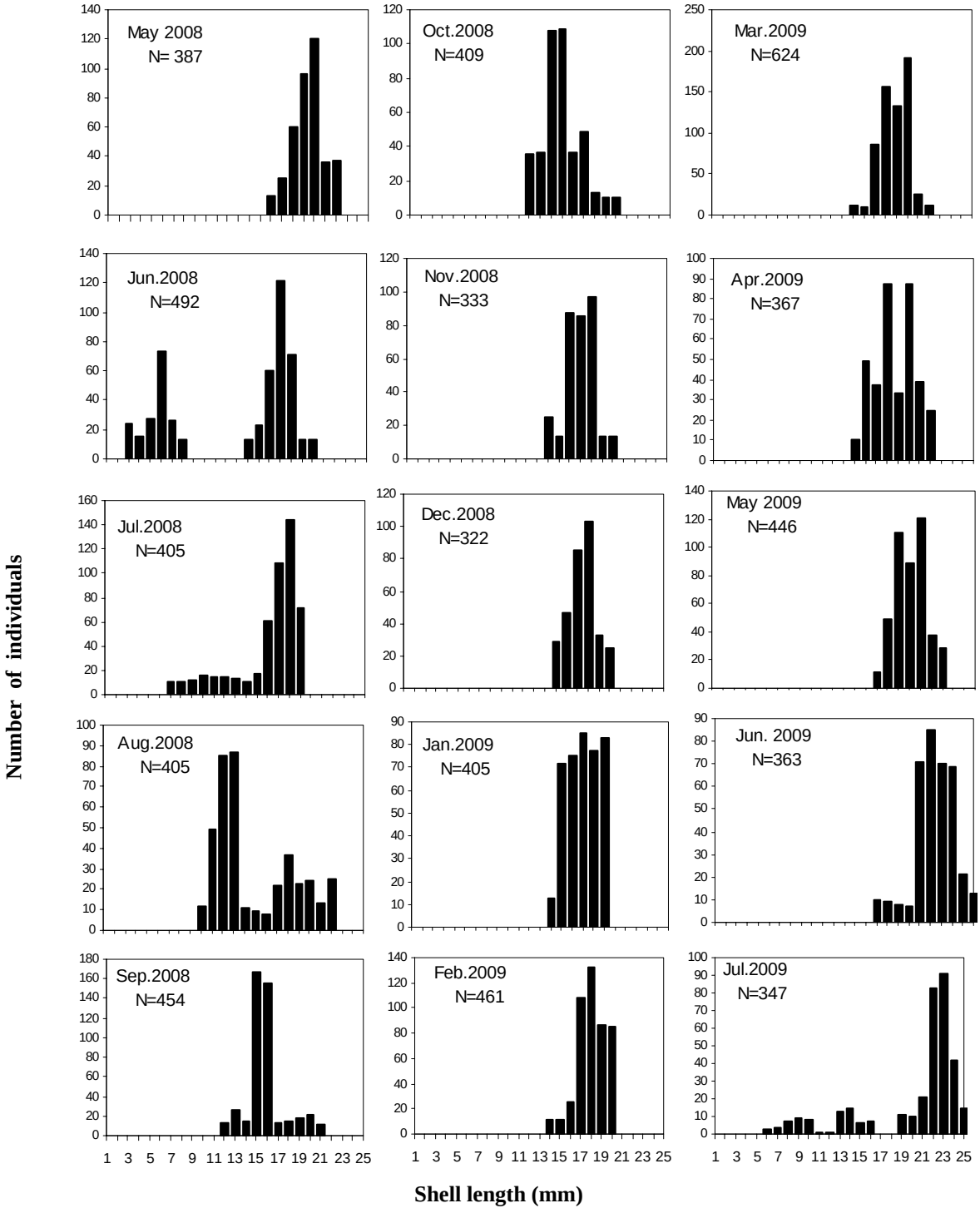
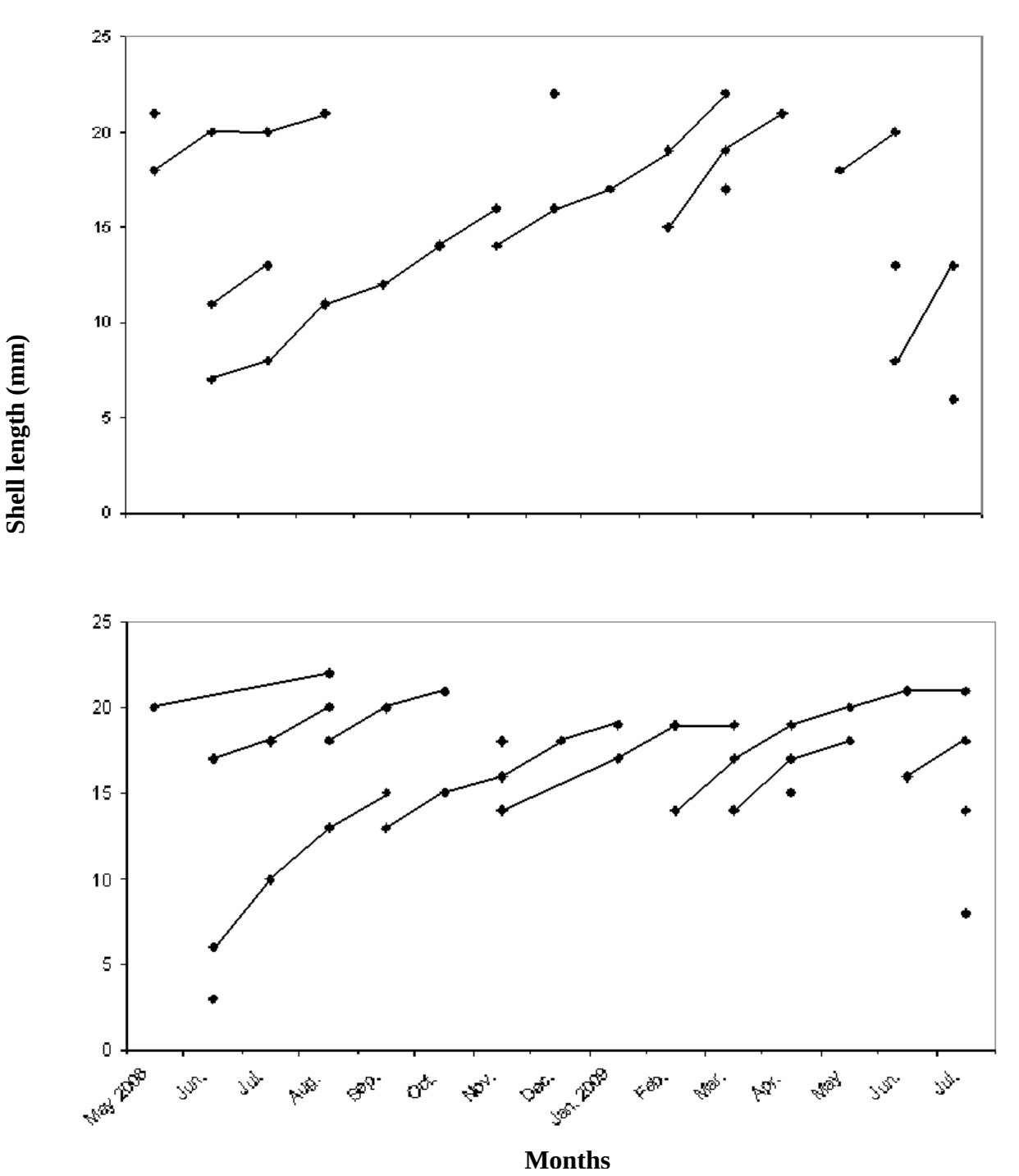


Fig. (5): Monthly size frequency histograms of *D. variabilis* samples collected from site II



Fig, 6 .The pattern of growth of the component populations of the *D. variabilis* at site I(a) and site II(b)

4. Discussion:

Donax variabilis is the dominant macro fauna on many of the exposed beaches of the south-east United States (Pearse *et al*., 1942). It found from Virginia south to Florida and round into the Gulf of Mexico to Texas (Ruppert & Fox, 1988). In the current study, *D.variabilis* was recorded for the first time in Egypt in the nearby region of the Maritime Damietta Port, there fore it considered as an invasive species from the Atlantic Ocean, where the larvae attached to commercial ships coming into port.

Distribution and density of clams are influenced by environmental changes, whether abiotic and biotic factors as well as to human activity. *D.variabilis* is more vulnerable to abiotic stressors than would be expected of invertebrates from habitats such as tide pools, mudflats or marshes (Grieshaber & Völkel, 1998).The number of *D.variabilis* per cubic meters increased at site I than at site II. The proximity of site II from the maritime port of Damietta increases the disturbances and movements of clams away either vertically or horizontally. Burrowing behaviour is an important adaptation (Brown & McLachlan, 1990 and McLachlan *et al*., 1995). Bivalves inhabiting all types of soft substrates are capable of burrowing easily, but bivalves living on exposed beaches must burrow rapidly and efficiently to avoid physical exclusion of individuals by waves or current action (Brown & McLachlan, 1990 and McLachlan *et al*., 1995). Physical properties of sediment, such as particle size, grain shape, water content and shear strength, affect the suitability of a substrate as a habitat, by influencing the burrowing behaviour and life habits of benthic species (Sanders, 1958 and Trueman, 1971).

D.variabilis, like many Donacidae, is noted for its mobility, and moves up and down the beach with the tide (Turner & Belding, 1957), and displays a pattern of sophisticated responses to waves and wave action (Ellers, 1995a,b). In addition, there is a seasonal cycle of movement, down into the shallow sub-littoral in fall and returning on to the beach as juveniles in late winter (Ruppert & Fox, 1988).

D.variabilis collected from the sampling sites increased during summer months and declined during winter. This similar to that happen, in laboratory experiments that for both adults and juveniles of *Donax*, burrowing time increased in lower temperatures (McLachlan & Young, 1982).

D.variabilis prefer water salinity slightly less than that of the sea, therefore it was collected from the area near the estuaries at New Damietta. Coastal and estuarine systems are highly productive areas that serve as nursery grounds for many marine species of commercial importance, widely distributed on the continental shelf (Beck *et al*., 2001 and Peterson, 2003).

D.variabilis distribution decreased with the increase in the concentration of nutrient elements. Hypoxic conditions owing to extremely high primary production and subsequent oxidative degeneration of organic matter (Van der Plas, 1999 and Fossing *et al*., 2000). With the increase in the density of *D.variabilis*, phytoplankton biomass reduced as a result of its consumption by these clams. Food webs of sandy beaches are mainly based on marine sources, such as phytoplankton, stranded algae, sea grasses and carrion (McLachlan & Brown, 2006).

Macroinfauna participating specially arthropods and other types of molluscs compete with clams, where as these animals' increased clams' movements to other places. Members of the genus *Donax* are commonly the main primary consumers in soft bottom communities, while they are in turn subject to predation by a wide variety of invertebrates, fish, birds and mammals (e.g. Luzzatto & Penchaszadeh, 2001; Peterson et al 2000 and Salaset al., 2001). Larger macrobenthic invertebrates burrow actively and include representatives of many phyla, but crustaceans, molluscs and polychaete worms are usually dominant and encompass predators, scavengers, filter- and deposit feeders (Defeo *et al*., 2009).

Ramon *et al*. (1995) estimation of the growth rate of *D.trunculus* in the western Mediterranean from an analysis of the length-frequency distributions, showed that there were two recruitments of clams to the population each year, one cohort was recruited during the winter whilst the other entered the population during the summer. Although spawning takes place during summer the season is long enough to show some intra seasonal variability in the gametes emission.

A unimodal pattern of recruitment between May and July was revealed in the present population, presumably owing to the measurement of larger individuals, settled earlier, by means of the mesh size utilized. Also the occurrence of a single annual recruitment has been found in the Atlantic populations (Ansell & Lagardere, 1980 and Guillou & Le Moal, 1980).

Results indicate that a mixture of biotic and abiotic factors mediates recruit abundance, with beach gradient being the most influential, followed by adult and juvenile abundances. The recognition and description of spatial patterns and their temporal dynamics are fundamental to understanding ecological processes that structure biological assemblages (Renshaw & Ford, 1984; Andrew & Mapstone, 1987; Volkaert, 1987; Jones *et al*., 1990;

Morrisey *et al*., 1992; Underwood *et al*., 2000 and Schoemana & Richardson, 2002).

On conclusion, *D.variabilis* is more susceptible to abiotic stressors and it is important concern for the environment in which they live in order to maintain production because of its importance to the people as food and for future culture.

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Egyptian Folk Art and its Significance as a Source of Symbolic Design Decorative Clothes Young Men and Women

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Abstract: The purpose of this study is to shed light on the importance of folk art as a national art, which should be with him to maintain the continuity by employing a selection of units of the Egyptian People and their meanings of symbolism in the decorative design of the T-shirt as a product commensurate with the youth of both sexes during the age (20 to 30 years), by identifying the views of all producers of clothes, textile and consumers in the proposed designs and the potential demand for purchase and implementation of a selection of them. The research samples contain 418 single distributed according to the research variables on the producers and the number (10) and intended them gentlemen producers of clothes for young people of both sexes and in particular the product T Shirts, and consumers are (408), and understood to mean members of the community of young men and young women aged (20 to 30 years) level of education between (high, medium, low), in order to know the views of samples of the research in the proposed designs and made the most important findings point to the as follows: -

1 - the best designs in accordance with the views of producers in the "appropriate decoration popular designs of the proposed" order is a design (V, IX, II, XIV, XI, and IV), due to the fact that these designs bear the character of the popular in contemporary more than Other designs, and then followed in the order designs (VIII, XIII, XV, XVI, and I), and comes at the end designs (X, VII, III, and XII).

2 - the best designs in accordance with the views of producers on "the possibility of the implementation and marketing of proposed designs," the order is the design, "IV, IX, XIV, I, VI, and VI," The reason for this is that these designs can be implemented by more than a method with low costs of production "In terms of raw materials, method of implementation of the decoration, lines run inside the factories," as it gives a higher percentage of profits as a result of consumer acceptance for, and then followed in the order designs, "XII, XIII, V, II, VII", and comes in the end designs "XI, X, XV, and VIII".

3 - There are significant differences between the mean scores of the views of consumers according to the research variables "in the appropriate technical designs proposed at the level (0.01) to the (female, age from" 25 to 30 "years, higher education).

4 - There is no statistically significant difference between the averages of the views of consumers according to the research variables "sex" in the extent of consumer acceptance of the proposed designs.

5 - There are significant differences between the mean scores of the views of consumers according to the research variables "age, level of education" in the extent of consumer acceptance of the designs proposed "at the level (0.01) for the (age of" 25 to 30 "years, higher education).

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Keyword: Egyptian folk art, symbolic meaning, decorative design, Clothing, young men and women

1. Introduction:

The folk art in any society is a manifestation of culture and an honest mirror reflect his ideas, including the beliefs, traditions, customs and its aspects of distinguishing either material or spiritual. The outcomes of the interaction of all these forces are formulated in the templates, unsentimental, and nourish the faith and strengthen the hearts and refine the humanitarian aspects of all. It also a language understood by all peoples and are affected by and have a key role and an imperative in building a civilization of human societies through "movement, line, shape, flat color, and suggestive words formulated in the tales and proverbs, poems, songs and melodies", which in reality are one and the building collected by unit membership one is the rights and abilities of the three "idea, share, and sentiments".

Folklore of any society have been affected and influenced other cultures, but retained by its components and meanings of the original as well as the creativity innate, which is in fact an expression of the nation and her personality. Which in all its forms a technical production has an authenticity innovative full of symbols and associated with history and myth and it is very close to life and society. In spite of the

importance of folk art, it is not diffuse adequately in the design and implementation of the clothing has been shown through a few of the studies and previous research, which dealt with popular icons and their meanings in the arts as diverse as the study (Demerdash, 1998), which employed the folklore in some artifacts to add aesthetic values of garments for women. (al-shourbagy, 2006), during his research aimed to find a relationship between popular icons as the value of art and aesthetic and functional design supplements upholstery fabrics printed, the acquisition of design dimension of the aesthetic and functional through the design of innovative new technologies. Youssef, 2006; benefited from the decoration People and their meanings is in the field of supplements clothing. Jaafar, 2008, analyzed folktales technically and aesthetically, and access to new thinking in the field of traditional clothing with symbols derived from folktales. Ismail, 2009, clarified the role of the functional and aesthetic trappings for the promotion of children of belonging to Egyptian trappings and employment in the furniture child room. This is prompting researchers to conduct a prospective study of young men and women to learn a preference for the clothes they were wearing and the nature of decorations that embellished, and find that many young people accept their pieces of clothes bearing the writings, or trademarks or decorations and charges do not reflect on the Egyptian identity. Here began the problem of current research as it is in the context of globalization and the era of information revolution took some nations lose their identity and presence and balance rapidly. This made the current study, trying to take advantage of the values the fine art of the units of the Egyptian People and their meanings symbolism, which form part of human culture and an essential element in the structuring of formative cultural, and try to highlight the national character of our art popular in the form of contemporary art for the dissemination of that culture in the young generation, through the creation of design motifs inspired by our popular and applied on the "shirt" in a way print view that through the survey it became clear tendencies of young men and women wearing T-shirts printed by the decorations and different graphics. Where, the producers of clothes young men and women acknowledged that the product t-shirt of pieces of clothes that can be applied to the decorations more than a method.

It was noted by researchers that, there is a tendency for some interesting research and previous studies highlighting the relationship between some of the arts and art of fashion design as an examination (Abdel-Majid, 2002), who studied primitive art as an entry point to see the Fine contemporary in the field of fashion design. Abdel-Majid, 2004, recruited innovative formulations of some elements of decoration in the Mamluk era of innovation designs of modern women bear the characteristics of the doctrine of abstract style design on the mannequin, and a study (Nur al-Din: 2009), which was inspired by works of art to inform the doctrine of abstract geometric design, "Suet-shirt" Men. Although all these studies of which have been associated with the idea of the current study, in part, only that it had agreed in the idea and the results obtained by the recommended research to sources of art and a variety of ways to open the field of artistic insights to further innovations in the field of fashion design as well as the deployment of the different arts through clothing.

It is clear from the previous view that the primary purpose of the present study is to shed light on the importance of folk art as a national art which, should be with him to maintain the continuity by employing a selection of units of the Egyptian People and their meanings of symbolism in the decorative design of the T-shirt as a product commensurate with the youth of both sexes through the ages of (20:30) in general, and to identify the views of both producers and consumers in the proposed designs and the potential demand for purchase, as the implementation of some of the proposals design that received the highest results through the views of the producers of clothing and textile hub your possibility of the implementation and marketing of proposed designs.

In this study shed light on the concepts of Egyptian folk art, classification, characteristics of folk art, decoration, signs and symbols of the fees, the procedural steps of the study, detailed presentation of the hypotheses of the study and discussion of results that have been reached through the statistical treatments and designs executed, the most important recommendations.

Concepts associated with the Egyptian folk art: -

Folk art Known as, the objects and ornaments that are made either for daily use or for decoration for special occasions such as concerts held for wedding and funerals. The folk art influenced by patterns of the group and the extent of their test, generation after generation, relying on the continuation of the social environment which is often in the rural people and neighborhood People in cities (CHLVERS, 1988), is defined in (Facilitator, 1965) Provided that the traditional art to the public, some arts and crafts a variety of others are merely an expression of art for the needs and the crowd of people and their feelings.

Was launched by the English writer, "William John Toms" on the popular arts the term "folklore" in the middle of the nineteenth century to

include the customs and traditions, myths and practices, and the word is folklore "folklore" of Old English and means the people or the people and the word "lore" of the Greek language, which means wisdom and become this term is the wisdom of the people supported and famous when he was adopted by the Assembly of folklore in English, which was founded in (1877), but during the twentieth century in Europe and the United States the development of this concept to include folk arts of all kinds of spiritual and material, and this concept is broader and more comprehensive because there is no difference between the tangible heritage and understanding of the spiritual element of popular culture and the basis (Al-Antil, 1987), and folk arts as defined by UNESCO experts as four catogries (sculpture and photography; arts; music, dance, and drama; literature and the arts verbal roots).

Properties of the Egyptian folk art: -

Both of Bassiouni, 1987, and Crow, 1999 defined the Characteristics of folk art in the following (folk art aesthetic does not know the individual because it is the art of the broad masses, a true mirror of society's culture and philosophy in life, art is level of culture of people, not to an individual, a combination of the symbols of life, legends and anecdotes, the clarity, transparency, purity and flatness, is not formal and away from the domination of the training on the symbols and shapes, and simplicity of language expression and stability, circulating with the generations and the link with good spatial, an indication of color directly with specific steps of the elements and symbols, the focus on story and myth combine between time and space, attention to color selections, hot and black, the importance of the religious dimension and its impact on folk art and the dimension of the political directives).

General features of the popular Egyptian heritage: -

folklore Characterized by (nobility, goes back to an old stages of the human history, a lively, spontaneous expression is not subject to the rules accepted in the art, depends on the cultural experience will move from one generation to another supported on the novel oral, the unit is a fabric one building collected by unit membership and a human one). (Bassiouni, 1987), (Crow, 1999)

Symbolism in folk art: -

Crow, 1999 and Gaber, 2005 demonstrated that is in the folk art is subject to the logic of surreal synthetic metaphysical, where classified symbols according to the trends of magical realism and alienation symbolic, and the popular icons like any work of art consisting of elements of plastic make it a subject where characterized by cohesion and harmony, and the significance refers to the sub-theme reflects the special spiritual reality.

Division of the popular motifs and the symbolic significance: -

1 - Trappings of the human body: (such as hand and eye for prevention, which are symbolic of envy, heart and arrow which symbolize love).

2 - Decorated with animal: such as (the lion, which symbolizes strength and protection, sentences which reflect the endurance and patience, deer symbol of beauty, the bird, which reflects the hope, the fish which refers to the goodness and hope, the dove that symbolizes peace and life)

3 - Floral decorations: (such as Palm, which reflect the goodness and life and steadfastness, cypress tree, which symbolizes the goodness and fertility).

4 - Geometric motifs: (such as parallel lines, which symbolize the flowing water, which reflects the triangle about the veil and the implications of magic and talismans, the crescent which reflects the life and Islam).

5 - decoration faith: like the sun, which reflects the life and growth and optimism, the mosque and minaret, which stands to the divine, the swastika, a Christian symbol that indicates a good omen if the branch at the end is going for the right and demonstrates the omen bad if the contrary, the bride that reflect the life and hope and purity, renewal and hold).

6 - Human units: such as (a form of women or girls which refers to the femininity and beauty, Knight, which reflects the strength and courage and the war).

7 – Written motifs: for example, words that reflect the wisdom and cues (such as companion by the way).

8 - Decoration color: (such as white, which reflects the purity and the feast, the black, which refers to the mourning and grief, blue, which reflects the cold, green as a symbol of tender, development and good, yellow indicates jealousy, the red cross of love). Figures (1.2, 3, 4.5, 6) examples of folk art decorations Egyptian

Search limits: - research is restricted to

1 - Employment vocabulary builder artistic decoration of the Egyptian People's signifier and signified the symbolic in the creation of fifteen design of a product T-shirt "t shirt" fit in with the youth of both sexes in the age group of (20 to 30) in sizes (s - m - l). The reason for choosing this piece of clothes is the conclusion reached by researchers through the survey, which have carried out on a sample of (young of both sexes, and producers), it was shown that, it the most common clothes for young people because of it is comfort and diversity in

use. This result made the authors going to take this piece of clothes as a model for the deployment of Egyptian popular culture arts.

Fig (1) Fig (2) Fig (3) Fig (4) Fig (5) Fig (6)

Figure (1) Combining form (the camel, the bride, horse, palm, palm tree, moon, star, eye, fish)
Figure (2) The form of combining (the dove, the veil, the sun, Al Ain, Palm, palm)
Figure (3) A girl form
Figure (4) Form shows (the lion, Knight, arrow, sword)
Figure (5) Illustrates the form of (Bride-born fish, Crescent)
Figure (6) Spica (Qansu, 1996), (http://www.islam on line.net / Arabic).

2 - Application of designs decorative innovative product t-shirt with style silkscreen "The Art of serigraph" It is a way to print, by preparing the design you want to print either manually or photography that represents the surface layout a special type of the fabric of follicular made of natural silk is used in this research, taut on a frame of wood, where the covers were silk fabric places is to be printed mediator is a port from cellulose, and used a much more this way because it is unique in its potential arts and technology excellence and accuracy of the details of which are difficult to obtain through the print the other, where it is practical way to obtain the values of technical aesthetic Court, could be put to use (add and drop, switch, overlay), the possibility of printing the number of colors or mixing effects and techniques of art is limited

2. Methodology: -

The current research followed the descriptive approach to explore the views of both producers and consumers in the proposed designs with the application through the implementation of a selection of them and that got the highest score.

Sample search: -

The sample consists of a number of (418) Single variables distributed according to the search for "producers, and consumers" The following table shows the distribution of the sample: -

Table (1) the distribution of the sample according to the research variables

Sample type	Number	%
Producer	10	3
Consumer	408	97
Total	418	100

Table shows the previous sample is distributed according to the research variables, which consisted of: -

- Producers: a number of (10) and their intended Gentlemen producers clothes for young people of both sexes and in particular product "t-shirt" to get to know their views on the proposed designs, which bear the character of Egyptian pop.
- Consumers: a number of (408), and understood to mean members of the community of young men and young women between the ages of (20:30) years the level of education between (low, medium, high) to identify the acceptability of the designs inspired by motifs popular Egyptian The following table shows the classification of consumers depending on the sample (sex, age and education level).

Table (2) classification of consumers depending on the sample (sex, age, education level)

Gender	Number	%	Age	Number	%	Level of education	Number	%
Male	173	42	(from 20: less than 25 years)	227	56	Low	84	20
Female	235	58	from (20:30) years	181	44	Medium	121	30
						High	203	50
Total	408	100	Total	408	100	total	408	100

As illustrated in table (2), that the proportion of males in the sample research has reached (42%) and the proportion of females was (58%), also turns out that (56%) of the sample ranged in age between (20 to less than 25) in general, while (44%) ranged of age (25 to 30) year, either for the education levels of the

sample reaching the proportion of people with low education (20%), while the percentage of those with intermediate education (30%), (50%) for higher education.

Research hypothesis:

The present research is testing the validity of the following hypotheses:-

1 - There are significant differences between the designs proposed in the "appropriate extent of the proposed design motifs popular," according to the views of producers.

2 - There are significant differences between the designs proposed in the "over the possibility of implementing the proposed design and marketing," according to the views of producers.

3 - There are significant differences between the mean scores of the views of consumers in the "appropriate technical designs of the proposed" depending on the research variables (sex, age, education level).

4 - There are significant differences between the mean scores of the views of consumers in the "over-acceptance of the proposed designs," according to the research variables (sex, age, education level).

Search Tools: -

1 - A questionnaire is open to youth of both sexes prefer to see young people wearing clothes that decoration and embellished.

2 - A questionnaire to get feedback from producers in designs inspired by the Egyptian folk art decorations.

3 - A questionnaire to find out the extent of consumer acceptance of the designs inspired by the Egyptian folk art decorations.

4 - Adobe Photoshop for the coloring of the proposed designs.

5 - Tools and materials used in silk screen printing and implementation of the shirt.

Steps to conduct research:

Steps included conducting current research on the following themes: -

The first axis:

study included exploratory have carried out by researchers to identify the direction of young men and women to choose their clothes and the nature of the decorations that embellished, and through the use of questionnaire open, consists of nine questions wave of young people of both sexes, confirmed its validity by presentation to a committee of experts of the arbitrators in the field of garments and textile to ensure the veracity of its content has been admitted validity of the application, where every young man or young woman to answer each question in a way an article from the reality of preference and the choice of a piece of clothes.

The second axis: include

1 – Collecting previous research and literature associated with the popular Egyptian art and its significance in symbolic and use a variety of areas, to know the location of current research of these studies and the similarities and differences among them.

2 - Determining the popular motifs used for inspiration in the present research, namely, (hand, eye, fish, camel, lion, pigeons, bird, sun, Palm, Knight and the spear, the veil in the form of a triangle, the girl, the words (such as companion by the way), colors (white, black, red, yellow, blue, green).

Axis III: included

1 - Distribution of selected motifs of folk art in the Egyptian producer T-shirt to show the different visions of the piece of clothes each time differently from the others. The authors have made fifteen designs determined as follows, to clarify this through technical analysis of the designs proposed: -

- the two designs (1) and (2): - a collectively of the various elements of popular icons between the elements of animal appeared in the shape of a lion, which symbolizes strength and protection, and the pigeon, which symbolizes peace, hope and life, and geometric shapes that have emerged in the form of triangles express the semantics magic and the Crescent, which symbolizes the beginning of birth, and used it as parallel lines and curly, which reflect the flow of water. The two designs are giving an imagine of the form of the village that appeared in the form of boxes stacked symbol of popular houses and towers of pigeons. The author have been used in the first design blue grade to give a sense of the dimensions of different terms of use dark color in the home of a sense of proximity to the light blue in birds to express flying in the sky and use a white background to highlight the idea. While, the second design has been to merge the two colors are green and black decorative design on a neutral background is gray.

-Design (3): - adopted the idea of design on the inscription, which was in the writing of such people in an irregular manner very similar to writings found on houses popular, has been used to highlight decorative units as popular as hand and eye which symbolize to prevent harm and the prevention of evil and envy two of the human motifs in addition to engineering units appear in the form of the screens, and triangles reflect numerology and hieroglyphs, was used to highlight this design color purple on gray background to highlight the clarity of the idea.

Proposed design (1)

Proposed design (2)

Proposed design (3)

The designs (4) and (5): - the strength of the idea of designs is the form of a woman or girl that point in the folk art of Egyptian femininity, beauty and fertility, highlighting the shape of the eye which symbolizes the prevention of envy with the overlapping of some floral and geometric to show the spirit of folk art, was adopted by the design idea to divide the spaces into different shapes and sizes measurements are distributed in parallel to bring the role of the viewer to see the shapes and complemented by a glance, as we see in design (4) the distribution of unit decorations on the chest area only and use a black on a white background to highlight the idea and emphasized, while in Design (5) units have been distributed in the form of rectangles of various sizes, including chest as a whole, and use the degrees of beige, red and green, a color inspired by the colors of the Egyptian People's decoration.

Design (6): - raised the idea of design of symmetry and uniformity to some of the shapes in order to emphasize the importance of the item public value of art, and adopted a decorative design on the use of decorations animal a fish that symbolizes the hope and reproduction, and units of humanity of a hand and eye which symbolize the prevention of envy and the prevention of harm, and geometric shapes of the shape of a triangle, which reflects the form of the veil as a focus of the work, and bars on the boxes inside the triangles, which symbolize the evil and abuse, as if to stop and the veil prevented harm, which reflect the curse of those tapes, which make the emergence of idea and the unity of form to achieve the objective functional designed for.

Proposed design (4)

Proposed design (5)

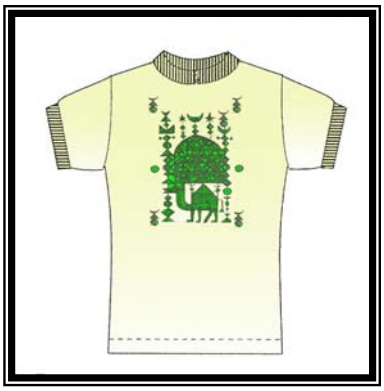
Proposed design (6)

Designs (7), (8): - built the idea of two designs on the character inspired by the folktales, Abu Zaid Hilali, who symbolizes the courage and the protection of sitting on a lion, so as to emphasize the idea of the strength and courage, have been distributed unit decorative design (7) in the chest and use her colors brown and blue, and to highlight the shape decorative been her frame in gray on the background color light yellow. Nevertheless, design (8) has been repeating unit decorative regularly on the product t-shirt as a whole using the colors of brown and light blue on a white background.

Design (9): - adopted the design concept to overlay its units decorative is the camel's back in engineering, and the dome and the Crescent, which symbolizes the birth and life, where the division of interior space for two units to various geometric patterns such as triangles opposite which stands for equality and justice, and circles that indicate to the divine, and squares that symbolizes balance, and use those colors brown and green alternately, and this diversity of lines and spaces has prominent elements and the unity of form, which together serve the purpose of functionality that was designed for.


Proposed design (7)


Proposed design (8)


Proposed design (9)

Design (10): - adopted the design on the distribution of motifs on a regular basis on the t-shirt as a whole in the form of columns, longitudinal, has been used where integration between plant motifs are palm fruit to express the good, decorated with human figures a stop, decorated with animal a fish, and geometric motifs on the disk of the sun as a symbol of sanctity, was also used colors inspired by the folk art of Egypt which is green, red and yellow.

Design (11): - From the elements of the symbols of popular distributed randomly and in different sizes and graduated from high to low, you may use the faithful fish, eyes, amulets and triangles and the dove in black on a red background so as to give unity to the design to serve the purpose of functionality that was designed for.

Design (12): - the strength of the idea of design is repeated decorative units painted in a geometric and divided spaces internal to the engineering units that are most important characteristic of folk art, and these units are repeated on a regular basis on an area of t-shirt as a whole and use the color black to highlight the designs decorative and clarity


Proposed design (10)


Proposed design (11)


Proposed design (12)

Design (13): - adopted this design on the distribution of units of brides born and fish sizes and different shapes, and dealt with in the abstract by dividing the area of internal units to and form various geometric patterns, and use the black and white alternately, achieving unity among the parts of the design as a whole.

Design (14): - From the elements of the environment of people who depend on integration between the natural elements of palms as a symbol of goodness and animal motifs are sentences which reflect the patience and endurance, and the Dove, which symbolizes hope and peace, and was used for the design of decorative color and one is maroon red on

the background of beige and to highlight the decorative aspect and emphasized.

Design (15): - Use of this design units geometric motifs overlap is in the forms of services complete and incomplete, lozenges, and triangles facing with the crescent and star that indicate optimism and determine the seasons and holidays, were distributed to these units in bulk on the t-shirt as a whole, has been used red and black, to highlight elements and confirmed to serve the functional purpose, which was prepared from futures design.

Proposed design (13)

Proposed design (14)

Proposed design (15)

2- Steps to build the questionnaire: -

a - questionnaire the views of producers in the textile and apparel sector

- In order to become acquainted with the views of producers, industry professionals in the field of clothes for young people of both sexes in designs inspired by the trappings of Egyptian folk art, and included the resolution on the two axes as follows: -

Axis I: appropriate decoration popular designs proposed falls below (6) statements.

The second axis: the possibility of the implementation and marketing of proposed designs, falls below (10) statements.

Thus, the total words of resolution (16) is under (48) degrees, and consists of balance-resolution three-estimate (OK, agree to some extent, reject), and by giving three degrees of acceptance, and two degrees of acceptance to a certain extent, degree and one for non-approved, also included on the questionnaire data at the beginning of her answer Screened.

-Psychometric transactions to identify the views of producers:

Believe resolution: researchers used two types of honesty and are as follows: -

Believe arbitrators: display resolution on a group of experts from the professors in the field of garments and textile, in order to verify the authenticity of the questionnaire and give feedback in terms of (the language of the themes and phrases, sequence and organization of axial resolution, appropriate words for each axis of that State, the sequence and organization statements of each axis), has recognized the validity of the application after making some amendments to the order of terms and adjust the wording of the axes.

Statistical truth:

Using the internal consistency between the degree of each axis and the total degree of the questionnaire is illustrated in the following table: -

Table (3) The internal consistency between the degree of each axis and the total degree of the questionnaire for producers

Themes	Correlation coefficient	Significance
Appropriate decoration popular designs of the proposed	0.852	0.01
The possibility of implementing the proposed design and marketing	0.708	0.01

Is clear from the above table that the values of correlation coefficients (0.852, 0.708), respectively, values statistically significant at the level (0.01), which shows the sincerity of the axes of the questionnaire.

The stability of resolution: The Calculation of stability through the (coefficient alpha, retail midterm) the following table illustrates this: -

Table (4): Reliability coefficient axis resolution for producers

Themes	Coefficient alpha	Mid-term retail
Appropriate decoration popular designs of the	0.768	0.842 - 0.727

proposed		
The possibility of implementing the proposed design and marketing	0.844	0.887 - 0.796
Questionnaire as a whole	0.806	0.864 - 0.760

Is clear from the table above that all transactions with alpha-and mid-term retail is the high values indicate the stability of the questionnaire

B - questionnaire the views of consumers about the proposed designs: the design of researchers Extension (5)

- Designed questionnaire to know the extent of acceptance by consumers (youth of both sexes) for the proposed designs and motifs inspired by the folk art of Egypt, and included a questionnaire on two axes as follows: -

Axis I: appropriate technical designs of the proposed falls below (15) words.

Axis II: consumer acceptance about the proposed designs, falls below (7) statements that measure all the positive trend. Thus, the total words of resolution as a whole (22) is under (66) degrees, and made resolution of the balance estimate three (OK, OK, to some extent, but OK), and by giving three degrees of OK, and two degrees of OK to a certain extent, degree and one for non-approved , also included on the questionnaire data at the beginning of her answer Screened.

- Psychometric transactions to identify the views of producers:

Believe resolution: researchers used two types of honesty and are as follows: -

Believe the arbitrators: the attention of the group of experts from the professors in the field of garments and textile extension (3) in order to verify the authenticity of the questionnaire and give feedback in terms of (the language of the themes and phrases, sequence and organization of axial resolution, appropriate words for each axis of that State, the sequence and organization of words each axis), all of whom have agreed to the validity of the application.

Statistical truth: using the internal consistency between the degree of each axis and the total degree of the questionnaire is illustrated in the following table: -

Table (5): The internal consistency between the degree of each axis and the total degree of the questionnaire for consumers

Themes	Correlation coefficient	Significance
Appropriate technical designs of the proposed	0.908	0.01
Consumer acceptance about the proposed designs	0.823	0.01

Is clear from the above table that the values of correlation coefficients (0.908, 0.823), respectively, a statistically significant values at the level (0.01) which shows the sincerity of the axes of the questionnaire.

The stability of resolution: The Calculation of stability through the (coefficient alpha, retail midterm) the following table illustrates this: -

Table (6): reliability coefficient axes resolution of consumer

Themes	Alpha Coefficient	Mid-term retail
Appropriate technical designs of the proposed	0.753	0.833 - 0.714
Consumer acceptance about the proposed designs	0.859	0.896 - 0.811
Questionnaire as a whole	0.829	0.877 - 0.781

Is clear from the table above that all transactions with alpha-and mid-term retail is the high values indicate the stability of the questionnaire

Axis IV: includes:-

1 - After you finish creating designs inspired by the trappings of Egyptian folk art building the two questionnaires which offer on the appointed research "producers and consumers," and to see how accepted it and select the best (6) designs received the highest grades in accordance with the views of the producers for their implementation and practical application to become realistic models suitable to put in the local and global markets for the deployment of Egyptian pop culture art through it.

3. Results and Discussion

a - First hypothesis states that: "There are significant differences between the designs proposed the appropriateness of decoration popular designs proposed in accordance with the views of the producers".

To verify the validity of this hypothesis was calculated analysis of variance to find the differences between the mean scores of designs proposed in accordance with the views of the producers and the following table illustrates this: -

Table (7) Analysis of variance of the average degree of the proposed designs in accordance with the views of producers in the "appropriate decoration popular designs of the proposed"

Source of variation	Sum of squares	Average squares	Degree of freedom	F value	Significance
Between groups	**1180.828**	84.**345**	14	44.**088**	**0.01**
Within groups	**258**.270	1.**913**	135		
Total	**1439**.098		149		

Is clear from the above table that the value of "F" was (44.088), a value statistically significant at the level (0.01), which indicates the existence of differences between the designs of Fifteen and in accordance with the views of producers in the "appropriate decoration popular designs of the proposed" To know the direction of significance, the test application (LSD) for multiple comparisons The following table illustrates this: -

Table (8) illustrated the followings: -

- The presence of statistically significant differences between the fifth design and the rest of the designs for the design at the level of the fifth (0.01). While no statistically significant differences between the fifth design and design at the level of the ninth (0.05) for the fifth design.
- The presence of over statistically significant between the ninth and the rest of the designs for the design at the level of the ninth (0.01), whereas no statistically significant differences between the ninth and Design II.
- The presence of statistically significant differences between the second design and the rest of the designs for the second design at the level (0.01), whereas no statistically significant differences between the second design, design XIV.
- The presence of statistically significant differences between the XIV and design the rest of the designs for the design at the level of the XIV (0.01), while no statistically significant differences between the XIV and design of both the IV and XI.
- The presence of statistically significant differences between the design XI and the rest of the designs for the design XI at the level (0.01), while no statistically significant differences between the design XI design and XIII at the level (0.05) for the design XI, while there is no statistically significant differences between XI and IV and VIII designs.
- The presence of statistically significant differences between the IV and the rest of the design, at the level of the fourth (0.01), while no statistically significant differences between the design of the IV and VIII and XIII designs.
- The presence of statistically significant differences between the VIII design and the rest of the designs, for the design at the level of the eighth (0.01), whereas no statistically significant differences between the design of both the VIII and XIII and XV.
- The presence of statistically significant differences between the design XIII and the rest of the designs for the design XIII at the level (0.01), while no statistically significant differences between the design the III and both I and X at the level (0.05) for the design the III, while there are no significant differences between the VI and XV.
- The presence of statistically significant differences between the XV design and the rest of the designs for the design XV at the level (0.01), while no statistically significant differences between the XV and both designs of I and XVI.
- The presence of statistically significant differences between the VI design and the rest of the designs, for the VI design at the level (0.01), while no statistically significant differences between the VI design and all of the designs I, VII and X.
- The presence of statistically significant differences between the I design and the rest of the designs for the first design at the level (0.01), whereas no statistically significant differences between each of the I design and the VII and X.
- The presence of statistically significant differences between the design X and the rest of the designs for the design at the level of X (0.01), whereas no statistically significant differences between the design X and design VII.
- The presence of statistically significant differences between the VII design and the rest of the designs for the design at the level of the VII (0.01), whereas no statistically significant differences between the VII and III designs.
- The presence of statistically significant differences between the III design and design XII for design at the level of the III (0.05).
- Clear from the foregoing that the best designs in accordance with the views of producers in the "appropriate decoration popular designs of the proposed" order is design (V, IX, II, XIV, XI, and IV), due to the fact that these designs bear the character of the popular in contemporary more than other designs, and then followed in the order designs

Table (8) Denote multiple comparisons of the fifteen designs determination in your "Tailor made popular decorations of the proposed design," according to the views of producers

Statement	Design 1 P=9.650	Design 2 P=14.450	Design 3 P=7.520	Design 4 P=12.060	Design 5 P=16.780	Design 6 P=9.730	Design7 P=8.600	Design 8 P=1.530	Design 9 P=15.310	Design 10 P=9.500	Design 11 P=6.270	Design 12 P=10.900	Design 13 P=13.234	Design 14 P=25.813	Design 15 P= 10.320
Design 1	-														
Design 2	** 4.800	-													
Design 3	** 2.130	** 6.930	-												
Design4	** 2.410	** 2.390	** 4.540	-											
Design5	** 7.130	** 2.330	** 9.260	** 4.720	-										
Design 6	0.080	** 4.720	** 2.210	** 2.330	** 7.050	-									
Design7	1.050	** 5.850	1.080	** 3.460	** 8.180	1.130	-								
Design8	**1.880	**2.920	**4.010	0.530	** 5.250	**1.800	**2.930	-							
Design 9	**5.660	0.860	**7.790	**3.250	**1.470	**5.580	**6.710	**3.780	-						
Design 10	0.150	**4.950	**1.980	**2.560	** 7.280	0.230	**0.900	**2.030	**5.810	-					
Design 11	**2.810	**1.990	**4.940	0.400	** 4.320	**2.730	**3.860	**0.930	**2.850	**2.960	-				
Design 12	**3.380	**8.180	**1.250	**5.790	**10.510	**3.460	**2.330	**5.260	**9.040	**3.230	**6.190	-			
Design 13	* 1.250	**3.550	**3.380	1.160	**5.880	**1.170	**2.300	0.630	**4.410	* 1.400	1.560*	**4.630	-		
Design 14	**3.584	1.216	**5.714	1.174	**3.546	**3.504	**4.634	**1.704	**2.076	**3.734	0.774	**6.964	**2.334	-	
Design 15	0.670	**4.130	**2.800	**1.740	**6.460	0.590	**1.720	1.210	**4.990	0.820	**2.140	**4.050	0.580	**2.914	-

**= significant at the level of (0.01)

* = significant when the level (0.05)

(VIII, XIII, XV , VI, and then I), and comes at the end designs (X, VII, III, and XII).

B - The second hypothesis states that: "There are significant differences between the designs proposed in the" over the possibility of implementing the proposed design and marketing, "according to the views of producers".

To verify the validity of this hypothesis was calculated analysis of variance to find the differences between the mean scores of designs proposed in accordance with the views of the producers.

Table (9) Analysis of variance of the average degree of the proposed designs in accordance with the views of producers on "the possibility of implementing the proposed design and marketing"

Source of variation	Sum of squares	Average squares	Degree of freedom	F value	Significance
Between groups	**5751.041**	410.**789**	14	42.**367**	**0.01**
Within groups	**1308.951**	9.**696**	135		
Total	**7059.992**		149		

It is clear from the above table that the value of "F" was (42.367), a value statistically significant at the level (0.01), which indicates the existence of differences between the designs of XV and in accordance with the views of the producers in "the possibility of the implementation and marketing of proposed designs" and to find out the direction of significance, the test application (LSD) for multiple **comparisons. Table (10) illustrates this: -**

Is shown from the table (10) as follows: -

- The presence of statistically significant differences between the IV and the rest of the design, design for design at the level of the IV (0.01), while no statistically significant differences between the IV and IX.
- The presence of statistically significant differences between the design and the rest of the IX designs for design at the level of the IX (0.01).
- The presence of statistically significant differences between the XIV and design the rest of the designs for the design at the level of the XIV (0.01), while no statistically significant differences between the XIV and design the I design at the level (0.05) for the design, the XIV.
- The presence of statistically significant differences between the I design and the rest of the designs for the I design at the level (0.01), while no statistically significant differences between the I design and design at the level of the VI (0.05) for the I design.
- The presence of statistically significant differences between the VI and design the rest of the designs for the VI design at the level (0.01).
- The presence of statistically significant differences between the III and the rest of the design, design for design at the level of the III (0.01), while no statistically significant differences between the III design and design at the level of XII (0.05) for the III design.
- The presence of statistically significant differences between the XII and design the rest of the designs for the design at the level of XII (0.01), while no statistically significant differences between the design, the XII and XIII design at the level (0.05) for the design XII.
- The presence of statistically significant differences between the XIII and design the rest of the designs for the design XIII at the level (0.01), while no statistically significant differences between the XIII and Design V Design.
- The presence of statistically significant differences between the V and design the rest of the designs for the design at the level of the (0.01).
- The presence of statistically significant differences between the II design and the rest of the designs for the II design at the level (0.01).
- The presence of statistically significant differences between the VII design and the rest of the designs, for the design at the level of the (0.01), while no statistically significant differences between the VII and XI designs at the level of (0.05) for the design VII.
- The presence of statistically significant differences between the design XI and the rest of the designs, for the design XI at the level of (0.01), while no statistically significant differences between the design XI and X design.
- The presence of statistically significant differences between the design X and the rest of the designs for the design at the level of (0.01), whereas no statistically significant differences between X and XV designs.
- The presence of statistically significant differences between the XV and VIII designs for design at the level of the (0.01).
- It is clear from the foregoing that, separate designs in accordance with the views of the producers in "the possibility of the implementation and marketing of proposed designs," the order is the design, "IV, IX, XIV, I, VI, and then the III," The reason for this is that these designs can be implemented by more than a

Table (10) Significance for multiple comparisons of fifteen determination in the possibility of implementing the proposed design and marketing," according to the view of producers"

Statement	Design 1 P=24.572	Design 2 P=16.089	Design 3 P=21.222	Design 4 P=28.860	Design 5 P=17.809	Design 6 P= 23.222	Design7 P=14.289	Design 8 P=10.622	Design 9 P=28.010	Design 10 P= 12.150	Design 11 P=13.031	Design 12 P=19.941	Design 13 P=18.681	Design 14 P=25.813	Design 15 P=11.460
Design 1	-														
Design 2	** 8.483	-													
Design 3	** 3.350	** 5.133	-												
Design4	** 4.288	** 12.771	** 7.638	-											
Design5	** 6.763	** 1.720	** 3.413	** 11.051	-										
Design 6	** 1.322	** 7.161	** 2.028	** 5.610	** 5.441	-									
Design7	** 10.283	** 1.800	** 6.933	** 14.571	** 3.520	** 8.961	-								
Design8	** 13.930	** 5.467	** 10.600	** 18.238	** 7.187	** 12.628	** 3.667	-							
Design 9	** 3.438	** 11.921	** 6.788	0.850	** 10.201	** 4.760	** 13.721	** 17.388	-						
Design 10	** 12.422	** 3.939	** 9.763	** 16.763	** 5.659	** 11.100	** 2.139	** 1.528	** 15.860	-					
Design 11	** 11.541	** 3.058	** 8.191	** 15.899	** 4.778	** 10.219	* 1.258	** 2.409	** 14.979	0.881	-				
Design 12	** 4.631	** 3.852	* 1.281	** 8.919	** 2.132	** 3.309	** 5.652	** 9.319	** 8.069	** 7.791	** 6.910	-			
Design 13	** 5.891	** 2.592	** 2.541	** 10179	0.872	** 4.569	** 4.392	** 8.059	** 9.329	** 6.531	** 5.650	* 1.260	-		
Design 14	* 1.241	** 9.724	** 4.591	** 3.047	** 8.004	** 2.563	** 11.524	** 15.191	** 2.197	** 13.663	** 12.782	** 5.872	** 7.132	-	
Design 15	** 13.112	** 4.629	** 9.762	** 17.400	** 6.349	** 11.790	** 2.829	0.838	** 16.550	0.690	** 1.571	** 8.481	** 7.221	** 14.353	-

**= significant at the level of (0.01)

* = significant when the level (0.05

method with low cost of production "in terms of raw materials, method of implementation of the decoration, lines run inside the factories," as it gives a higher percentage of profits as a result of consumer acceptance for, and then followed in the order designs, "XII, XIII, V, II, VII," The designs in the end "XI, X, XV, and VIII".

C - The third hypothesis states that: "There are significant differences between the mean scores of the views of consumers in the" appropriate technical designs of the proposed "depending on the research variables (sex, age, education level)."

To verify the validity of this hypothesis has been applied "T" test to calculate the differences between the mean scores of the views of consumers in the appropriate technical designs of the proposed depending on the study variables (gender, age, education) and the following tables illustrate this: -

Table (11): Significant differences between the mean scores of the views of consumers in the appropriate technical designs proposed for the variable "sex and age"

Variable		Mean	Standard deviation	Sample	Degree of freedom	T (value)	significance
Gender	Male	22.**4624**	5.**41692**	173	406	24.**758**	0.01 for females
	Female	36.**3574**	5.**73487**	235			
Age	From 20:25 year	24.**3392**	5.**96482**	227	406	24.**705**	0.01 For age (25: 30) years old
	From 25:30 year	38.**1492**	5.**12888**	181			

It is clear from the above table that the value of "T" in the variable sex was (24.758), a value statistically significant at the level (0.01) in favor of females, with an average degree of female (36.3574), while the average scores of boys (22.4624) and because the reason for this to that males at the age of (20:30), who have the embargo, and prudence, patience, where use planning in thinking towards the new subjects, unlike females who accept what is a new self-fulfillment and a sense of independence and uniqueness amid peers and this is consistent with what was called for by (Rimawi, 1994), who pointed out that there are differences between the sexes in the immersion emotional response to new subjects, especially art in favor of females, and attention to thought processes and organization in favor of males and deal with new issues caution in favor of males, and females are more interested in social activities and artistic, while males are more interested in professional activities, and supports this outcome study (Abu Nile, 1988), which reached to the existence of significant differences between the sexes in the social and economic values, preferences and technical aspects of the personal in favor of females was more responsive in the previous aspects.

As indicated above table also that the value of "T" in the variable age was (24.705), a value statistically significant at the level (0.01) for the Age of "25 to 30" years old, with an average degree of Age of "25 to 30" years old (38.1492), while the average degree of Age of "20 to less than 25" years old (24.3392) The reason for this is that young people in the Age of (25: 30) are stable in the decisions they always do their utmost in the quest for a positive push to the formation of identity, making them feel that the proposed designs and inspired by the folk art of Egypt is an important factor for the consolidation of that heritage, which is the mirror of true community in which they live, where they reflect the ideas of society and culture with its beliefs and traditions and respects distinguishing whether material or moral, and this is consistent with what was called for by (Abu Hatab, Sadiq, 1990) that the young man in this age group tends to the identification and dissemination, as it appears has the responsibility towards the social institutions and the tendency to spread information about the features of his home.

Table (12): Analysis of variance of the mean scores of consumers in the appropriate technical designs of the proposed "depending on the variable of education"

Source of variation	Sum of squares	Average squares	Degree of freedom	F value	Significance
Between groups	**16158.230**	8079.**115**	2	53.**002**	**0.01**
Within groups	**61734.748**	152.**431**	405		
Total	**77892.978**		407		

It is clear from the above table that the value of "F" was (53.002), a value statistically significant at the level (0.01), which indicates the existence of differences between the views of consumers in the appropriate technical designs of the proposed depending on the level of education, and knowledge of the direction of significance was applied test (LSD) for comparisons multiple the following table illustrates this: -

Table (13) test (LSD) for multiple comparisons

Statement	Lower education p=**8.976**	Moderate education p= **12.165**	Higher education p=**17.719**
Lower education	-		
Medium education	** 3.**189**	-	
Higher education	** 8.**743**	** 5.**554**	-

It is clear from the above table there are significant differences between higher education and intermediate education and low education for the benefit of higher education at the level (0.01), and there are significant differences between middle school education and low education for middle school education at the level (0.01), due to the fact that knowledge acquired by the young men and women through higher education occur a change in behavior and trends, and increase the inclination to enrich the culture in various fields, and thus directly affect the decision-making, and make them accept what's new in different areas without restriction or hesitation reverse educational levels, at least, such as education medium and low, with less knowledge of individuals and thus less opportunities to increase their own culture with what goes on around them, topics, and supports this result was highly commended by (Abdel-Fattah, 1985) that education is essential for life change attitudes of individuals and give them self-confidence, making them make decisions quickly without restrictions.

D - The fourth hypothesis states that: "There are significant differences between the mean scores of the views of consumers in the" consumer acceptance of the proposed designs, "according to the research variables (sex, age, education level)."

To verify the validity of this hypothesis has been applied test "T" to calculate the differences between the mean scores of the views of consumers, along with consumer acceptance of the proposed designs depending on the study variables (gender, age, education) and the following tables illustrate this: -

Table (14): Significant differences between the mean scores of the views of consumers in the extent of consumer acceptance of the designs proposed for the variables "Age and education"

Variable		Mean	Standard deviation	Sample	Degree of freedom	T (value)	significance
Gender	Male	14.**6705**	4.**8334**	173	406	1.**543**	Not significant
	Female	13.**9787**	4.**79549**	235			
Age	From 20:25 year	11.**2115**	3.**09857**	227	406	23.**954**	0.01 For age (25: 30) years old
	From 25:30 year	18.**1105**	2.**60533**	181			

It is clear from the above table that the value of "T" in the variable sex was (1.543), a value that is statistically significant and due to the fact that researchers in the course of invented the proposed designs take into consideration the selection and distribution units, decorative Egyptian in line with the views of young men and women about their preference for clothing which make the results of accepting an almost equal. While, the value of "T" in the variable age (23.954), a value statistically significant at the level (0.01) for age (25 to 30) years with an average degree of age (25 to 30) years old (18.1105), while the average the degree of age (20 to less than 25) years (11.2115) and the reason for this is that young people in the age group of (25: 30) years old enjoy the stability of the resolution without restrictions or worry, as they tend to love the beauty and adornment, making them care about and focus on personal appearance is different from the peer, thus making them proposed designs that reflect the character of the Egyptian to see them have it with a different appearance from other decorations other raised in the domestic and international markets, and that young people in this age understand the importance of Egyptian Folklore and how to maintain him in various ways may be the clothing of them to ensure their continuation and spread across different generations, and this is consistent with what was called for by (Abu Hatab, Sadiq, 1990) that young people are at the stage of (25: 30) have developed a so-called clash of roles which have a grave effect in the formation of identity and, if successful young people in determining their identity after persistent efforts to avoid ambiguity and confusion, it is transmitted in a "sound towards the deployment of different cultures."

Table (15): Analysis of variance to the mean scores of consumers in the extent of consumer acceptance of the proposed designs, "according to the variable of education"

Source of variation	Sum of squares	Average squares	Degree of freedom	F value	Significance
Between groups	**3968.124**	1984.**062**	2	34.**814**	**0.01**
Within groups	**23081**.**249**	56.**991**	405		
Total	**27049**.**373**		407		

It is clear from the above table that the value of "F" was (34.814), a value statistically significant at the level (0.01), which indicates the existence of differences between the views of consumers in the extent of consumer acceptance of the designs proposed depending on "the level of education, and to find out the direction of significance was applied test (LSD) for multiple comparisons The following table illustrates this: -

Table (16) test (LSD) for multiple comparisons

Statement	Lower education p=**655.22**	Moderate education p= **24.405**	Higher education p=**37.310**
Lower education	-		
Medium education	** 1.**750**	-	
Higher education	** 14.**656**	** 12.**905**	-

It is clear from the above table there are significant differences between higher education and intermediate education and low education for the benefit of higher education at the level (0.01), while no statistically significant differences between middle school education and low education for middle school education at the level (0.05), and the reason for this is that education Higher in the various fields multiple positive effect on the attitudes of individuals and their interests and their decisions and make them enjoy the confidence and focus with what was happening around them changes in social, cultural and art and as such, increases the sense of individuals focusing on identity and interest in maintaining boiling Egyptian Folklore, which reflects the culture of the nation, and less attention to the low level of education that makes young people do not realize the importance of heritage or identity of the Egyptian culture as a result of lack of knowledge of the importance of the spread of folklore.

Axis V: selection and implementation of a selection of proposed designs

After analyzing the results of questionnaire, each of the "producers and consumers," it appears that there is a difference in the results of a pivotal questionnaire producers in priority order proposed designs, as shown by the difference in preference designs proposed for the youth in terms of (sex, age, education level), and as such tended researchers to agree with the views of producers in preference proposed designs depending on "the possibility of the implementation and marketing of proposed designs," The reason for this is possible to carry out those pieces of clothes with low-cost due to the small size of units, decorative printed designs (IV, IX, XIV, I, VI, and III), resulting in savings in raw silk printing and thus less product prices of clothes when placing on the market. Which invited the authors to select those designs to be cut implemented is as follows (construction engineering for the T-shirt motifs selected a scale of "1:10" , parts of the Pattern, the basic raw materials and assistance, silk printing applications, operational phases, and finally view the samples after the implementation of the youth of both sexes).

1 - Construction and engineering for T-shirts and motifs used in the application at a scale of "1:10", and parts of the Pattern: -

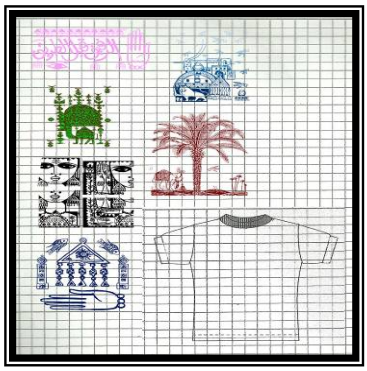


Figure (7) Construction engineering for the T-shirt motifs

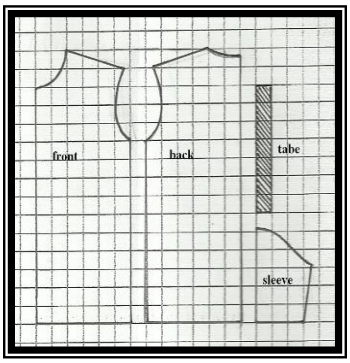
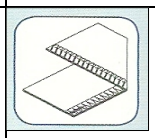
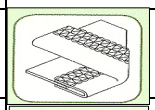
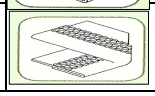


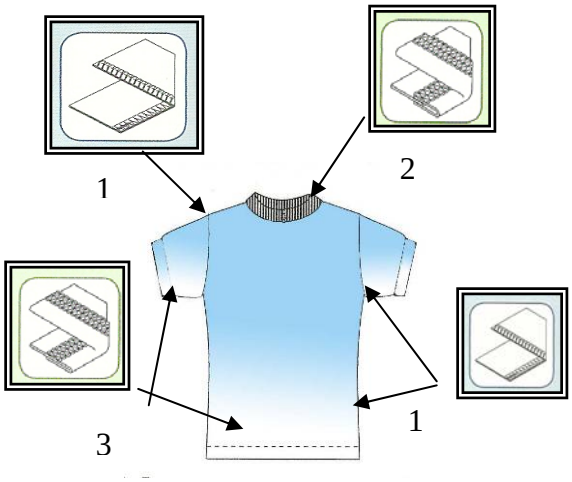
Figure (8) Pattern parts model t-

- The basic raw materials and assistance used in the implementation: - Knitwear Single Jersip Barcola (90% Cotton, 10% Lycra, and yarn 100% Paul ester).

3 - Applications silkscreen printing: - After you make the process of storytelling comes the stage of printing on a part forward in the designs proposed by the preparation of decorative design on the raw silk, stretched on a wooden frame, is used to paste "water base" in print and then pass the samples on "dryer" dried twice in a row at a temperature of "180 ° C", the duration of time "40" seconds at a time, in the proposed designs (1.9, 14), the proposed design (4) were given the texture of velvet by the addition of "solid" to the basic dough and then pass the object after printing on the impact of machine you are brocade sprayed powder which gives the effect of marigold, dried and then passed on three consecutive times at a temperature of "180 °C", the duration of time "40" seconds at a time, the proposed design (3) were given the appearance of a prominent "foam", by adding a paste foam to paste the basic rate of "50%", and after the printing process leaves a portion forward the publication for a day to dry and then passes on the dryer twice in a row at a temperature of "180 ° C", each time a period of time "40" seconds, and finally proposed design (6), which was given the brilliant look through the development of aluminum foil stained above the print and pass on the compressor "bress" strong pressure "6" bar and the temperature of "220 ° C "for" 10 "seconds and then left to cool and then tends paper aluminum.

4 - Different stages in the operation of structural design for the T-shirt used in all designs proposed after the printing process: -

Process description	The form of machine used	Stitch type and number	A stitch
Knitting pace of the shoulder, Knitting quantum slot armpit, weaving together the footsteps of the quantity and pace of side-shirts		Overlock stitch 504	
Installation of the tape in the neck slot		Overlock stitch 514	
End of the tail, and pagan quantum		Stitch coverage 406	



5- View the samples after the implementation of the sample youth of both sexes in order of preference of the attention of the producers and specialists in the feasibility and marketing: -

Port design (1), the design of the proposal (4)

Port design (2), the design of the proposal (9)

Port design (3), the design of the proposal (14)

Port design (4), the design proposal (1)

Port design (5), the design of the proposal (6)

Port design (6), the design of the proposal (3)

4. Research Recommendations:

In light of what has been exposed from the results of researchers recommend the following: -

A - To shed further studies and research that highlight the relationship between Egyptian folk art and art design fashion "for women, men, and children"

B - Take advantage of the popular arts in contemporary arts tradition is not as common now, but take advantage of their assets and their movements and solutions to various technical positions.

C - The establishment of university chairs in all universities and institutes to study different aspects of the Egyptian People's Arts for dissemination and consolidation of foreign universities like which is concerned with a study of folk arts.

D - Attention to the establishment of local and international exhibitions in the field of clothing design inspired by the folk arts of Egypt.

E - Attention to the establishment of libraries and documentaries to save all information related to folk arts of Egypt and through the assistance of the status of folk art and clothing and textile departments in colleges specialized in the Egyptian universities to work in scientific journals on all aspects of folk art to be deployed inside and outside the country.

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12/2/2010

Uncertainty determination of correlated color temperature for high intensity discharge lamps

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Abstract: Color temperature is a description of the color of light sources. The chromaticity coordinates of the light source lying on the Planckian locus which is called (Commission Internationale de l'Eclariage, referred to as CIE) CIE diagram and the source has color temperature (in Kelvin) equal to the blackbody temperature of the Planckian radiator. For light sources that don't have chromaticity coordinates that fall exactly on the Planckian locus but lie near it. In this case the chromaticity coordinates of such sources can be representing by correlated color temperature (CCT). Uncertainty of Correlated Color Temperature (CCT) or **(T_{cp})** for high intensity discharge lamps (HID) is derived from (u, v) color coordinates. The method of the International organization for standardization (ISO) Guide is applied by Gardner to drive analytical expression for uncertainty in **u** and **v** chromaticity coordinates and an uncertainty in CCT for few Kelvins can be achieved. The color temperature standard achieved with the uncertainty is. ± 11.48 K for mercury lamp, ± 3.44 K for sodium lamp and ± 6.4 K for metal halide lamp).
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http://www.americanscience.org.

Key words: lamp, correlated color temperature, Uncertainty and luminous flux.

1. Introduction:

Color temperature is a characteristic of visible light that has important applications in photometry science (calibration and lighting), photography, videography, publishing, manufacturing, and other fields. The color temperature of a light source is the temperature of an ideal black-body radiator that radiates light of the same chromaticity as that light source. The temperature is usually stated in Kelvin (K). It is directly related to Planck's law and Wien's displacement law. The CIE color coordinates are derived by weighting the spectral power distribution (obtained by using a spectroradiometer). the chromaticity coordinates are usually given by normalized coordinates x and y. The (x, y) coordinates are called the chromaticity coordinates. (1) The CCT of a light source, also expressed in Kelvins, is defined as the temperature of the blackbody source that is closest to the chromaticity of the source in this case the CIE 1960 (Uniform Color Space) UCS (*u*, *v*) system is used .(2)

A "modified uniform chromaticity scale diagram" suggested, based on certain simplifying geometrical considerations where (u, v) chromaticity coordinates was used instead of (x,y) . This (u, v) chromaticity space became the CIE 1960 color space, which is still used to calculate the CCT. (3). Higher color temperatures (5,000 K or more) are cool (blueish white) colors, and lower color temperatures (3,000 K or lower) warm (yellowish white through red) colors. For incandescent lamp is called color temperature but for flourescent and high intensity discharge lamps is called Correlated color temperature. (4)

In physics and color science, the Planckian locus is the path or locus that the color of an incandescent black body would take in a particular chromaticity space as the blackbody temperature changes. It goes from deep red at low temperatures through orange, yellowish white, white, and finally bluish white at very high temperatures. (5)

In this work we have to calculate the uncertainty in u, v and CCT for one high pressure mercury lamp has symbol W1, one high pressure sodium lamp has the symbol W2, and one metal halide lamp has the symbol W3.

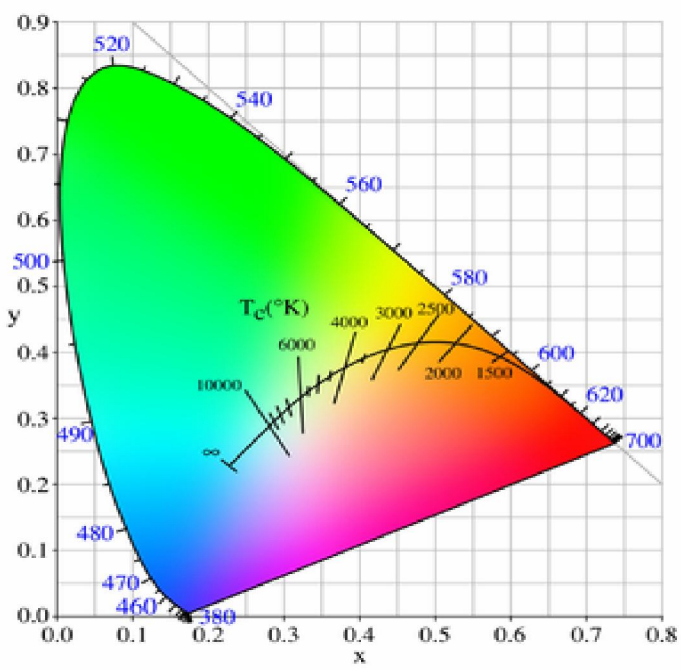

Figure 1.The CIE 1931 x,y chromaticity space, also showing the chromaticities of black-body light sources of various temperatures (Planckian locus), and lines of constant correlated color temperature.

2. The experiment technique

The measurements of CCT and u, v were done by HR 2000 spectroradimeter.

The spectroradiometer system is made up of several elements:

- input optics, (a source or sources, with power supplies and electrical measuring equipment).
- Polychromator (monochromator)/array detector,
- Data acquisition system (electronics for measuring detector output quantity combined with a data processing system).

The spectroradimeter ocean optics HR 2000

Irradiance uncertainty: 4.7%

Figure 2. Spectroradiometer measurements where illuminant A is used to take as reference spectrum borrowed from manual (6) .

In the present work we choose the one lamps of high pressure mercury 125 Watts, one lamp of high pressure sodium 150 watts and one lamp of metal halide 150 watts

Such lamps have CCT from warm (≈ 2200 K) to cool light (≈ 6500 K). In the spectroradimeter measurements irradiance is total radiant flux incident on an element of surface divided by the surface area of elements in W/m^2. (7) Before any work the lamps should be seasoned until the photometric and electric characteristics remain constant. In the present work the HID lamps must be seasoned for ***100*** operating hours and should be cycled ***11*** hours on and one hour off. The metal halide and high pressure sodium lamps should be stored in the same position as seasoned. (8)

3.Theoretical background

3.1 The uncertainty of (u, v)

The uncertainty in u is

$$u_c(u) = \{ (u-4)^2 \Sigma u_c^2(E_i) x_i^2 + u^2 [225 \Sigma u_c^2(E_i) y_i^2 + 9 \Sigma u_c^2(E_i) z_i^2] + 30u (u-4) \Sigma u_c^2(E_i) x_i y_i + 6u (u-4) \Sigma u_c^2(E_i) x_i z_i + 90 u^2 \Sigma u_c^2(E_i) y_i z_i \}^{1/2} / (\Sigma E_i x_i + 15 \Sigma E_i y_i + 3 \Sigma E_i z_i).$$

And similarly

$$u_c(v) = \{9(5v -2)^2 \Sigma u_c^2(E_i) y_i^2 + v^2 [\Sigma u_c^2(E_i) x_i^2 + 9 \Sigma u_c^2(E_i) z_i^2] + 6v (5v-2) \Sigma u_c^2(E_i) x_i y_i + 6 v^2 \Sigma u_c^2(E_i) x_i z_i + 18v (5v-2) \Sigma u_c^2(E_i) y_i z_i \}^{1/2} / (\Sigma E_i x_i + 15 \Sigma E_i y_i + 3 \Sigma E_i z_i)$$ (9)

Correlated color temperature CCT:

The CCT of a general source is defined the temperature of the nearest point on the Black-body locus.

The standard uncertainty u_c**(T)** in CCT is given by

$$u_c(T) = (\partial T/\partial u)^2 u_c^2(u) + (\partial T/\partial v)^2 u_c^2(v) + 2r_{uv} (\partial T/\partial u) (\partial T/\partial v) u_c (u) u_c (v).$$ (1)

Where r_{uv} is the correlation coefficient between u and v and

$$\partial T/\partial u = - 5918.47 + 9.69941 T - 0.00958899 T^2 + 1.88114x10^{-6} T^3 - 1.67343x10^{-10} T^4 + 5.42081x10^{-15}.$$

$$\partial T/\partial v = - 385.70 + 8.40689 T - 0.00362952 T^2 + 3.71034 x10^{-8} T^3.$$

The correlation coefficient between u and v is given by (1) is

$$r_{uv} = \Sigma (\partial u/\partial E_i) (\partial v/\partial E_i) u_c^2(E_i) / [\sqrt{} \Sigma (\partial u/\partial E_i)^2 u_c^2(E_i) \sqrt{} \Sigma (\partial v/\partial E_i)^2 u_c^2(E_i)]$$ (9)

x_i, y_i and z_i are color matching functions (description of a color by the spectral concentration of a radiometric quantity such as radiance or radiant power as a function of wavelength) from 360 nm to 770 nm and obtain from standard table . Radiant power is total emitted by a light source per unit time. (7)

Gardner obtains the uncertainty in CCT derived directly from systematic and random components of the spectral irradiadiance values. (10)

4. Results and discussions:

By setting the lamps at their nominal voltage at distance one meter from input fiber (optics). The u,v and CCT data of each lamp obtained from the computerized spectroradimeter, tabulated in tables (1-4). In table 1 the values of u,v and CCT for each lamp. In table 2 the values of uncertainty of u,v and their squares. In table 3 we obtained the uncertainty of CCT . Finally in table 4 the values of operating voltage, current and watt for each lamp. We found that photometrically and electrically the W1 is the high uncertainty in CCT for high pressure mercury lamp, the high pressure sodium lamp has lower uncertainty and W3 for metal halide lamp is intermediate. Gardner (9) uses this method for calculating CCT for a high pressure sodium lamp reaching uncertainty of CCT for this lamp as 3.1 K assuming an uncertainty of spectral irradiance $u_c (E_i)$ is 0.01 but we measure experimentally the uncertainty of spectral irradiance of spectroradimeter is $u_c (E_i) = 4.7\%$.

5. Conclusion:

- For the first time in Egypt experimentally determination of the uncertainty of CCT for high intensity discharge lamps.
- The lamps under investigation may use as standard lamps for correlated color temperature in national institute for standards (NIS).
- By using the uncertainty for CCT we can obtain the uncertainty for mismatch factor, which is very important for calculation of luminous flux uncertainty.

Table 1. The values of CCT and u and v were obtained by using the spectroradimeter.

Lamps	CCT	u	v
W1	6036	0.236	0.327
W2	2200	0.32	0.361
W3	6306	0.235	0.333

Table 2. The values of uncertainty of u and v and their square

Lamps	$u_c(u)$	$u_c(v)$	$u_c^2(u)$	$u_c^2(v)$
W1	0.03	0.018	0.000888	0.000324
W2	0.046	0.018	0.002116	0.00032761
W3	0.015	0.009	0.000228	8.14506E-05

Table 3. The values of uncertainty of CCT for lamps

Lamps	$\partial T/\partial u$	$\partial T/\partial v$	$(\partial T/\partial u)^2$	$(\partial T/\partial v)^2$	Uncertainty of CCT (Kelvin) ±K
W1	-29488.2	-17849.6	8.7E+08	3.2E+08	11.48
W2	-8476.06	-376.64	71843513	141858	3.44
W3	-31058.5	-19833	9.65E+08	3.9E+08	6.4

Table 4. The values of Current, volt and power of the lamps

Lamps	Volt (V)	Current (A)	Power (W)
W1	119	1.18	125
W2	91	1.71	133
W3	97	1.87	149

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Genotoxic Effects of Acrylamide in Adult Male Albino Rats Liver

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Abstract: Background: Acrylamide is a common chemical which is used in both industrial and laboratory processes. It is formed in heated starchy foods especially potato products. Aim of the work: The aim of the present study was to clarify the possible involvement of genotoxic mechanisms in acrylamide-induced hepatotoxicity by measuring the role of cytochrome P450 2E1 (CYP2E1) gene protein and mRNA in rats intoxicated with acrylamide and recording the DNA changes in their hepatic tissues by the *in vivo* alkaline single cell gel electrophoresis (Comet assay).
Material and Methods: Thirty mature male albino rats were used in this study. Rats were classified randomly into three groups; the first group daily received 50 mg/kg acrylamide orally for 21 days. The second group received twice the previous dose (100 mg/kg) by the same route and duration and the third group was administered distilled water and kept as control. Results: The results revealed that, acrylamide caused marked alterations in animal behaviour and mortality % in both treated groups which reached 30% (in the first group) and 40% (in the second group) . Acrylamide elicited a highly significant increase in serum AST and ALT, while a significant decrease of total protein, albumin and globulin levels were recorded. Acrylamide caused down regulation of both CYP 2E1 protein and its mRNA expression concomitant with a dose dependent significant increase in number of DNA single strand breaks. Histopathological investigation revealed necrotic and degenerative changes in the liver of acrylamide treated rats.
Recommendation: Acrylamide exposure either occupationally or dietary must be restricted. In addition to, raising awareness of people about its hazards.

[Khlood M. El- Bohi, Gihan G. Moustafa, Nabela I. El sharkawi and Laila M. E. Sabik. **Genotoxic Effects of Acrylamide in Adult Male Albino Rats Liver.** Journal of American Science 2011; 7(1):1097-1108]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Acrylamide, Glycidamide, Genotoxicity, CYP2E1, Comet assay .

1. Introduction:

Contaminants are a vast subject area of food safety and quality and can be present in our food chain from raw materials to finished product *(Erkeoglu and Baydar, 2010).* Acrylamide (ACR) is one of the most important contaminant in the environment, which was shown to be a neurotoxicant, reproductive toxicant and carcinogen in animals *(El-Assouli, 2009)* .

ACR is an alpha, beta- unsaturated vinyl monomer of poly acrylamide (conjugated) reacted molecule. The co-polymers and polymers of ACR have a wide range of applications, it is used in paper manufactures, waste water treatments and as soil stabilizers, in addition, it is used worldwide to synthesize polyacrylamide. Both polyacrylamide and ACR have numerous applications in cosmetic industries, plastic and aesthetic surgeries, ophthalmic operations, oil recovery processes and other industrial and laboratory processes *(Klaunig and Kamendulis, 2005 & Asha et al., 2008 and Schwend et al., 2009).*

Dietary ACR is largely derived from heat – induced reactions (Maillard reaction) between the predominantly amino group of the free amino acid precursor asparagine and carbonyl groups of glucose and fructose during heat processing (baking and frying) of plant-derived foods such as potato fries and cereals. Orally consumed ACR is absorbed into the circulation then distributed to various organs, and reacts with DNA, neurons, hemoglobin, and essential enzymes *Baum et al.,(2008) and Rayburn and Friedman , (2010),* causing several toxic effects as animal carcinogen and germ cell mutagen *Ghanayem et*

al., (2005) , also human neurotoxicant and suspected carcinogen (*Klaunig and Kamendulis , 2005 and Nuno et al., 2008).* ACR is not genotoxic by itself but becomes activated to its primary epoxoide genotoxic metabolite glycidamide (GA) via epoxidation *Baum et al.,(2008), by* CYP2E1 which leads to the formation of GA-DNA and hemoglobin adducts *(Ghanayem et al., 2005)*.

Nowadays appreciable amounts of ACR are formed in western diets which extensively invade our markets, this prompted renewed interest in its potential toxicity.

So the aim of this study was to clarify the possible involvement of genotoxic mechanisms in ACR-induced hepatotoxicity by measuring the role of CYP 2E1 (gene protein and its mRNA) in the liver of rats intoxicated with ACR and recording the DNA changes by the *in vivo* alkaline single cell gel electrophoresis (Comet assay).

2. Material and Methods

ACR compound purity is 99% and purchased from Sigma Chemical Company. It is a water-soluble vinyl monomer (*Shan et al., 2006).*
ACR synonyms: 2- propenamide; acrylic acid amide; acrylic amide; acrylamide monomer; acrylamide; propenoic acid amide ; vinyl amide ; ethylene carboxamide
Molecular formula: C_3H_5NO
Chemical formula : $CH_2CHCONH_2$
Chemicalstructure:CH_2=CH-C-NH_2 *(Ghanayem, et al., 2005).*
O

Animals and dosing

Thirty mature male albino rats with an average body weight ranging from 160-180 g were obtained from the Animal Research Unit of the Faculty of Vet. Medicine Zagazig University. Animals were kept in metal cages during the whole experimental period under hygienic conditions, fed on well balanced ration and provided with water *ad- libtum*, through the experiment. Rats were divided into three equal groups the first group daily received 50 mg/kg ACR by oral gavage for 21 days, the second group received twice the previous dose (100 mg/kg) by the same route and duration , *(Sumner et al. ,1999& 2003).* And the third group was administered distilled water and kept as control.

Clinical signs and mortality percentage (%) were recorded along the experimental period. At the end of the experiment blood samples were collected from medial canthus of the eyes of all male rats in plain tubes for serum separation according the method of Renwick (1989). Serum samples were kept at -20°C till analysis. Then the animals were sacrificed, tissue samples from the livers of both treated and control groups were taken and immediately preserved in liquid nitrogen till RNA extraction and semi- quantitative RT-PCR analysis and Comet assay. Another liver samples were preserved at -80°C till western immuonoblotting analysis.
For histopathological study, liver samples preserved in 10% neutral-buffered formalin.
Biochemical analysis

Serum samples were analyzed for estimation of alanine aminotransferase (ALT) and the aspartate aminotransferase (AST) activities *(Bergmeyer,et al. 1978)*, total proteins *(Henry, and Harper, 1964)* and albumin *(Doumas et al. 1971),* were determined . Serum globulin was calculated by substraction of albumin from total protein.

Microsomal preparation

The liver tissues were homogenized in 3 volumes of 1.15% potassium chloride solution and centrifuged at 9,000 g for 20 min. The supernatant fraction was centrifuged at 105,000 g for 70min. The washed microsomes were then suspended in 0.1M potassium phosphate buffer, pH7.4 *(Omura and Sato 1964).* Microsomal protein concentrations were determined by the method of *Lowry (1951)* using bovine serum albumin (Sigma Chemical Co.) as a standard.

Western immunoblotting analyses of CYP2E1 protein

Liver microsomes (5mg protein) were electrophoresed (Mini-Protean II, Bio-Rad Lab., Richmond, CA) through 10% Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) slab gels *(Towbin et al., 1979).* Resolved proteins were transferred to nitrocellulose Trans-Blot membranes (Bio-Rad Laboratories, Hercules CA). The blots were stained with Ponceau S to confirm that protein content is approximately the same in all lanes. Filters were soaked in phosphate buffer solution, pH7.5 (PBS) to remove the Ponceau S and were incubated overnight in PBS containing 5% dried skimmed milk and 0.1% Tween-20, to block excess protein binding sites.

The membranes were then incubated with a primary goat polyclonal anti-rat CYP2E1 antibody (Gentest Co., Woburn, MA), detected with horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminiscence reagents (Amersham Pharmacia Biotech, Ltd, Buckinghamshire, UK), and visualized. The CYP2E1 content was quantified by analysis densitometrically scanned using NIH image software 1.61.

RNA extraction

Total RNA was isolated from 50mg of liver using Trizol reagent (Life Technologies Inc, Grand Island, NY, USA). Briefly liver tissue samples were homogenized in 1ml of Trizol then 0.3ml of chloroform was added to the sample. The mixtures were then shaken for 30sec followed by centrifugation at 4°C and 15,000g for 20min. The supernatant layer were transferred to a new set of tubes, and an equal volume of isopropanol was added to the samples, shacked for 15sec and centrifuged 4°C and 15,000g for 15min. The RNA pellets were washed with 70% ethanol. RNA was dissolved in DEPC water. The prepared RNA was checked by electerophoresis, and showed that the RNA integrity was fine, and then it was further checked by measuring the optical density (OD) on specterophotometer. The OD of all RNA samples were 1.7 to 1.9 based on the 260/280 ratio.

Semi-quantitative RT-PCR

A mixture of 5µg of total RNA and 0.5ng oligo dT primer in a total volume of 24µl sterilized ultra-pure water, was incubated at 70 ºC for 10min and then removed from the thermal cycler and completed to 40µl with a mixture of 8 µl (5X) RT-buffer, 2µl 10mM dNTP, 2µl DEPC water, and 2µl of reverse transcriptase (Toyobo CO., Ltd., Osaka, Japan) and incubated in the thermal cycler at 30 ºC for 10min, 42ºC for 1h, and 90 ºC for 10min. For PCR, 1µl aliquots of the synthesized cDNA were added to 20µl of a mixture containing sterilized ultra-pure water, 2µl of PCR buffer, 2µl of dNTP (2.5mM), 0.3µl of sense and anti-sense primers (10µM) and 0.1µl of Taq polymerase (Takara, Kyoto, Japan). Specific CYP2E1 primers were designed according to *Gonzalez et al. (2003),* the sequence: GAAAAAGCCAAGGAACACC (sense) and GCAGACAGGAGCAGAAACA (antisense) as published PCR For semi-quantitative RT-PCR assays, a co-amplification approach was used with glyceraldehyde-3-phosphate dehydrogenase (G3PDH) as the control gene. Rat liver and the PCR conditions were the same as described above, except that 10pmol G3PDH primers (sense): TGAAGGTCGGTGTGAACGGATTTGGC and (antisense): CATGTAGGCCATGAGGTCCACCAC. Amplification was initiated by denaturation of 1 cycle at 95ºC for 1min followed by denaturation at 94ºC for 1min, and annealing at the proper temperature for 1min then extension at 72ºC for 1min for the proper number of cycles for each gene using a DNA thermal cycler (BioRad, Hercules, CA, USA). The samples were finally incubated for 7min at 72ºC after the last cycle of amplification. The amplified PCR products were separated by electrophoresis through 1-1.5% agarose gel. Bands of cDNA were stained with ethidium bromide and visualized by ultraviolet illumination. The CYP2E1 and G3PDH contents were densitometrically scanned using NIH image software 1.61. G3PDH mRNA levels were used for the correction of CYP2E1 mRNA expression as endogenous genes; the ratio between CYP2E1 and G3PDH was determined by densitometry.

Comet assay

Small piece of the hepatic tissue was collected and placed onto a small Petri dish with ice-cold mincing solution (Ca^{2+}- and Mg^{2+}-free HBSS containing 20 mM EDTA and 10% DMSO). The viability of the liver cells was indirectly determined by analyzing the comet images after electrophoresis *Endoh et al, (2002).* The liver samples were cut into smaller pieces, using a disposable microtome razor blade, and the solution was aspirated. Then, a fresh mincing solution was added and the liver samples were minced again to finer pieces. Resulting cell suspensions were collected and filtered (100 µm nylon mesh). All samples were stocked on ice in appropriate conditions to avoid light until the comet assay procedures. The quantity of liver cells in the cell suspensions was determined in Giemsa-stained smears.

The Comet assay was performed under alkaline conditions according to a previously described standard protocol *Collins and Dunsinka, (2002),* Briefly, an aliquot of 5 µl of each prepared hepatic cell suspension was mixed with 120 µl of 0.5% low melting point agarose at 37°C and

layered onto conventional microscope slides, precoated with 1.5% normal melting point agarose. The slides were placed overnight in freshly prepared cold lysing solution (1% Triton X-100, 2.5 mM NaCl, 0.1 mM Na_2EDTA, 10 mM Tris with 10% DMSO, pH 10.0) and then in a horizontal electrophoresis cube with alkaline electrophoresis solution (0.3 M NaOH, 1 mM Na_2EDTA, pH >13) at 4°C for 20 min. The electrophoresis was performed at 25 V and 300 mA for 20 min. After electrophoresis, the slides were washed twice for 5 min in neutralizing buffer (0.4 M Tris-HCl, pH 7.5), fixed for 5 min in absolute alcohol, air-dried, and stored at room temperature. In order to evaluate extremely low molecular weight DNA diffusion, two slides from each animal were removed after lysis procedure, rinsed with neutralizing solution, fixed and air-dried, and stored until analysis.

Immediately before analysis, the DNA was stained with 50 µl of 20 µg/ml ethidium bromide. The slides were examined with a 40X objective lens with epi-illuminated fluorescence microscopy (Olympus-Bx60, excitation filter: 515-560 nm; barrier filter: 590 nm) attached to a color CCD video camera and connected to an image analysis system (Comet II, Perspective Instruments, UK). Coded slides were scored blindly and 100 hepatic cell images were randomly analyzed for each animal (50 images per slide).

The Comets were analyzed by a visual scoring method and computerized image analysis. The comets were classified into five categories, defined as types 0, 1, 2, 3 and 4 - where 0 indicates no or very low damage, 1, 2 and 3 indicate low, medium and Long DNA migration, respectively, and 4 indicates apoptotic or necrotic DNA migration. Based on the extent of strand breakage, cells were classified according to their tail length in five categories, ranging from 0 (no visible tail) to 4 (still detectable head of the comet but most of the DNA in the tail). The following formula *Liu et al, (2002)* [was] used to calculate scores in which N is the number of cells in each category (e.g. N3 is the number of cells in category 3).

Score= (N0+N1+2xN2+3xN3+4xN4)/ (N0+N1+N2+N3+N4)

Experiments were done in duplicate and repeated at least twice.

Histopathological examination

Liver specimens were routinely processed and sectioned at 4-5um thickness. The obtained sections were stained with H&E according to *Horobin, and Bancroft , (1998).*

Statistical analysis

The results were analyzed using the Statistical Package of Social Science (SPSS) version 10 software. Analysis of variance, one way (ANOVA) for comparison between more than two groups. Least significant difference (LSD) for multiple comparison *(Norusis, 1997).*

3. Results:

Clinical signs:

Administration of ACR to male rats resulting in marked alterations in behaviour, revealing nervous manifestations (abnormal neurobehavior) in the treated groups as ataxia, increased landing of the limbs, weakness of the muscles, general emaciation. The severity of the clinical signs was dose and time dependant as these manifestations appeared on the 7th and 12th days of high and low ACR treated groups respectively.

Mortality and post-mortem picture:

Mortalities started at 3rd and 9th day of administration in high and low dose treated groups which reached 40% and 30 % respectively by the end of the experiment. Post-mortem (P.M.) lesions of either dead or sacrificed rats revealed generalized enlargement and paleness of body organs.

Serum biochemical parameters

There were a highly significant changes between mean values of (AST, ALT, total protein, albumin and globulin) in all treated groups all over the period of the study by ANOVA. By LSD, there were a highly significant increment in serum AST and ALT activities on comparing each treated group with the control and with each other. On the other hand each treated group revealed a highly significant decrement of total protein, albumin and globulin levels comparing with control group and with each other, except for globulin, which showed non significant changes on comparing both treated group (Table 1).

Expression level of CYP2E1 protein

On measuring the expression level of CYP2E1, the immune-blot of CYP2E1 protein revealed that, its gene protein in ACR treated rats was significantly down regulated and which was dose dependent, (Fig. 1A-B). Similarly a significantly down regulated mRNA expression of CYP2E1 was recorded in comparison to control group (Fig. 2A-B).

Comet assay

The role of ACR on the direct DNA single strand (ss) breaks was evaluated with Comet assay which could detect DNA ss breaks in hepatocytes after treatment. A statistically significant increase of the number of DNA ss breaks was evident with both examined concentrations of ACR and this increase was dose dependent (Fig. 3)

Histopathology findings

The examined livers of ACR treated rats with a dose of 50 mg /kg b.wt. Showed mild reversible degenerative changes characterized by cloudy swelling or hydropic degeneration of some hepatic cells, hypotrophied Kupffer's cells together with dilated and congested blood vessels and hepatic sinusoids beside numerous bile ductules (Fig. 4). Some portal areas exhibited edema and proliferative biliary epithelium with round cell infiltration. While ACR treated rats with a dose of 100 mg /kg b.wt. showed mild necrotic changes in the hepatic parenchyma represented by granular eosinophlic cytoplasm and karyolysis of some nuclei together with portal mononuclear cell infiltration (Fig.5). Moreover, round cell infiltration could be seen in portal and interstitial tissues beside telangiectiasis in some hepatic sinusoids.

Table 1. Effects of low and high dose of acrylamide administration on serum ALT,AST, total protein, albumin and globulin of male albino rats after 21 days by ANOVA and LSD tests.

Groups / Parameters	Control	ACR low dosed group (50mg/kg)	ACR high dosed group (100mg/kg)	P
AST (U/L)	34.6±2.3	42.8±1.9**a**	58.6±3.4**ab**	*<0.001
ALT(U/L)	23.8±1.7	32.2±2.2**a**	37.6±1.6 **ab**	*<0.001
Total protein(gm/dl)	6.9±0.3	5.3±0.6**a**	4.5±0.3 **ab**	*<0.001
Albumin(gm/dl)	4.1±0.1	3.2±0.4**a**	2.3±0.2 **ab**	*<0.001
Globulin(gm/dl)	2.8±0.2	2.1±0.2 **a**	2.2±0.1 **a**	*<0.001

* : Significant of ANOVA. Significant of LSD:

a : P < 0.001 when each treated group compared with the control.

b : P < 0.001 when ACR group(100mg/kg) compared with ACR group (50mg/kg).

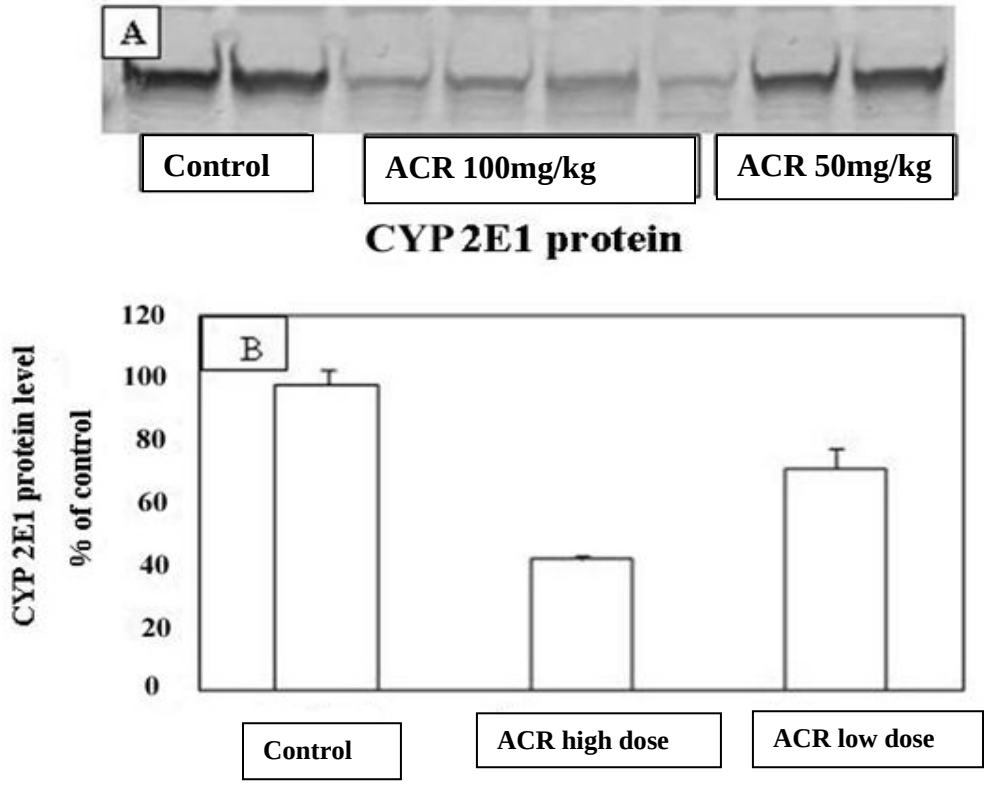

Fig.1 (A-B): Effects of acrylamide administration on expression levels of CYP2E1 protein, ACR was orally administered daily for 21 days by two dose levels 50 and 100mg/kg.
*p < 0.05 was considered statistically significant compared to control group.

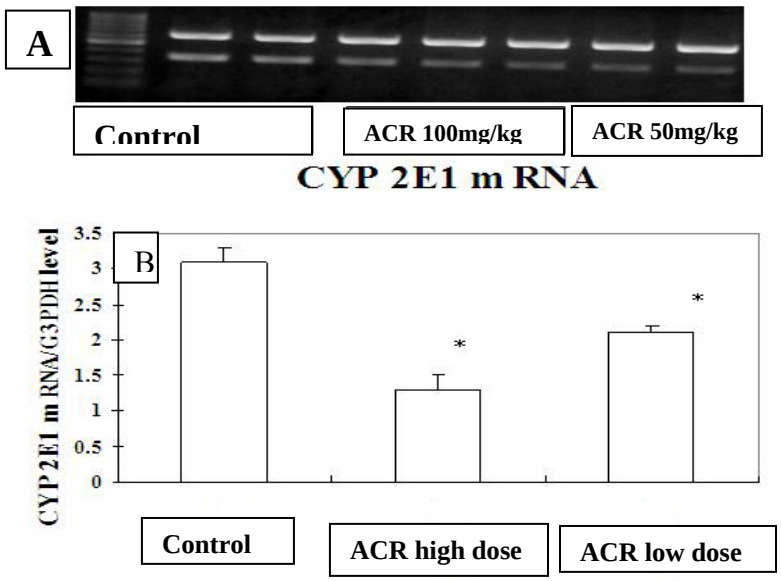

Fig. 2(A-B): Effects of acrylamide administration on Semi-quantitative CYP2E1 mRNA expression in the liver of control and acrylamide treated rats.
ACR was orally administered daily for 21 days by two dose levels 50 and100mg/kg. The values of CYP2E1 expression were corrected to G3PDH expression levels.
*p < 0.05 was considered statistically significant compared to control group

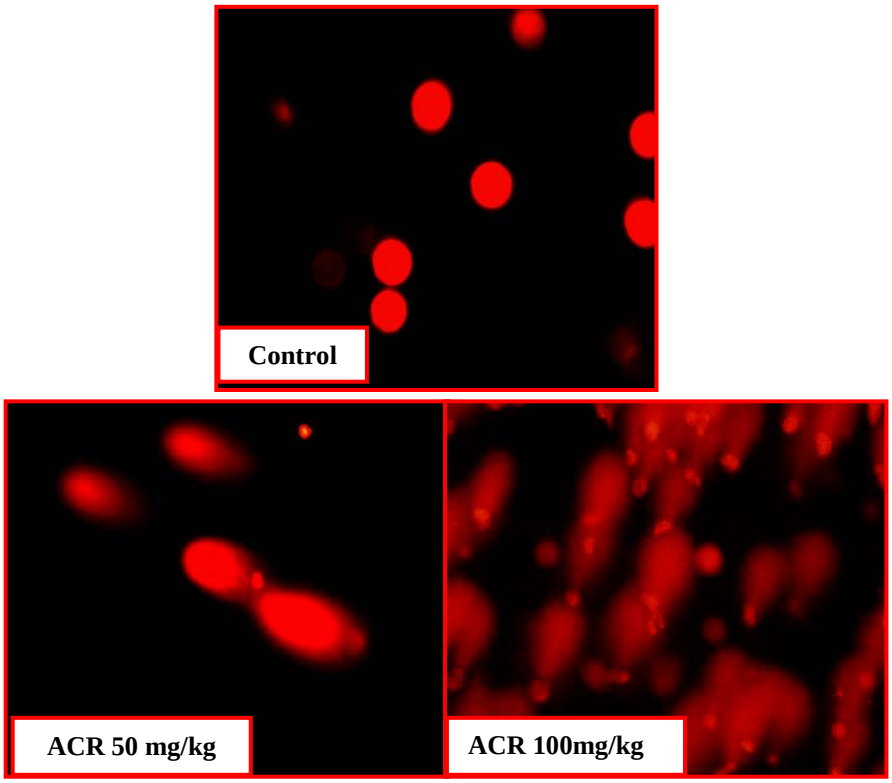

Fig. 3: Comet images of liver cells / PBS treated cells (Control group); ACR 50mg /kg daily for 21 days; ACR 100mg /kg daily for 21 days

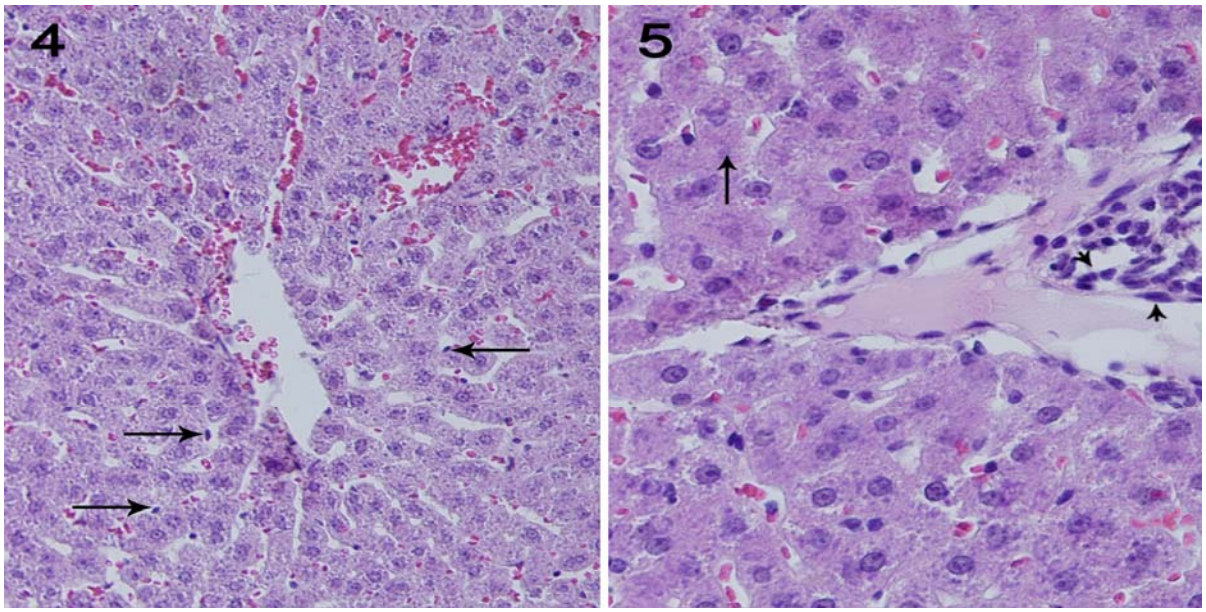

Fig. 4: Section in liver of adult male rat orally administered acrylamide daily at dose of 50 mg/kg b.wt for 21days showing degenerative changes characterized by cloudy swelling or hydropic degeneration of some hepatic cells, hypotrophied Kupffer cells (arrow) (H&E X 300) .

Fig. 5: Section in liver of adult male rat orally administered acrylamide daily at dose of 100 mg/kg b.wt for 21days showing mild necrotic changes in the hepatic cells (arrow) with focal mononuclear cells infiltration (head of arrow) in portal area (H & E x 1200).

4. Discussion:

In the current study, the administered doses of ACR were high compared with that estimated in cooked food which is as high as 70 µg per day (Tareke *et al.*, 2002). However to clarify the effect of ACR on CYP2E1 expression in the present study, we administered ACR to rats at 50and 100mg/kg.

The results of the present study revealed that marked abnormal neurobehavior which was dose and time dependant. These findings were parallel with *LoPachin (2000)* who determined the time of onset and development of neurotoxicity which were observed on day 11 after 50 mg/kg after ACR treatment. Hind limb dysfunction and abnormal gait recorded in the present work, coincide with Shukla *et al.,(2002)*who found that exposure of rats to ACR caused hind limb paralysis in 58% of the animals on day 10 , they attribute these findings to ACR neurotoxicity.

The mortalities that observed in the current study may be attributed to high dose of ACR administrated to rats, ACR neurotoxicity that causing hind limb dysfunction which lead to inability to get food , in addition, ACR may caused alterations in thirst and hunger regulation centres in hypothalamus *(WHO, 1985).*

There was a significant increase in serum AST and ALT activities, which was dose dependent. These results are similar to those recorded by *Chinoy and Memon ,(2001)& Yousef and El-Demerdash (2006)* in serum and plasma of mice and rats respectively post ACR intoxication.

These results confirmed by the hypothesis that recorded by *Chinoy and Memon ,(2001)* who attributed the significant increase in serum AST, ALT levels to the bipolar nature of ACR , where the CH2=CH part may undergo hydrophobic interactions while the CONH2 part can form hydrogen bonds with the cell components. This property may enhance its ability to alter the cell membrane structure and make the parenchymal cell membrane of liver more permeable, thereby causing the active retention of enzymes and making them appear first in the extracellular space and then in the blood. The previous changes were confirmed by histopathological findings.

Both biochemical and histological findings may be attributed to protein degradation which manifested by the low level of total protein, albumin and globulin in our result. The obtained results were found to be supported by the results of *Asha et al. (2008)* who reported that there were steady decreases in hepatic protein level with higher doses of ACR which can be resulted from retarded protein synthesis, or to change in protein metabolism or to the leaking out of protein reserves from hepatocytes. ACR molecule has two reactive sites, viz, the conjugated double bond and the amide group which can conjugate with the -SH group of a sulfur containing amino acids and α-NH2 group of a free amino acid. The above scenario can explain the unavailability of few amino acids for protein synthesis. Further being an electrophilic compound ACR can bind with proteins which can make them undetectable.

CYP2E1, a superfamily of hemoproteins involved in the metabolism of numerous xenobiotics with unrelated chemical structures comprises several isoforms with overlapping substrate specificity. Many of these substrates form reactive intermediates, thus becoming potent toxicants, mutagens or carcinogens. Among of them is CYP2E1, an isoform involved in the biotransformation of several small organic chemicals, including ACR and many others *(Jasso et al, 2003).*

Treatment of male albino rats with both doses of ACR for 21 days cause down regulated hepatic CYP2E1 which postulate that ACR requires CYP2E1-associated bioactivation producing GA causing liver injury. The immune-blot of CYP2E1 revealed that, the protein level of CYP2E1 in ACR treated rats was significantly down regulated and this inhibition was dose dependent. This observation is in accordance with the kinetic data, which showed that CYP2E1 is the rate- limiting factor of metabolic activation of ACR to GA *(Tareke et al., 2002, HSDB, 2003& PHS, 2004).* The down regulation of CYP2E1 gene protein was accompanied by reduction of the expression of CYP2E1 mRNA in a dose – dependent manner in comparison to control group . This down regulation may be due to direct cytotoxicity of GA which leads to reduction in the expression of CYP2E1 protein and mRNA due to inhibition of its transcription from the damaged hepatocytes. Our results are supported by the findings of *Naoki et al., (2011)* which revealed that the down regulation of CYP2E1 may indicates and confirm its role in the metabolism of ACR as it is predominantly active at high ACR concentrations. Previous findings have shown that ACR and its metabolite GA have affinity to bind with DNA, causing abnormalities in its structure which affect transcription and ultimately protein

synthesis *(Dearfield et al., 1995, CERHR, 2004 & Husoy et al., 2005).*

The present study revealed that there was a significant increase in the number of hepatic DNA ss breaks which was dose dependent.

Our results coincide with *Gamboa da Costa et al.,(2003)* Who found that several adducts of GA with the purine bases of DNA have been described as supralinear dose–response relationship which appeared in DNA of liver, lung and kidney of mice treated with ACR which are consistent with saturation of oxidative biotransformation of acrylamide at higher doses. This may be attributed to the alkylating properties of ACR (alpha, beta – unsaturated vinyl monomer) or its reactive metabolites GA which cause cytotoxic effects *(Schwend et al., 2009).*

On the same context , *Nicole et al., (2005)* stated that ACR possess clastogenic and mutagenic properties *in vivo and in vitro* due to its reactive metabolites GA which act as ultimate mutagenic agent.

5. Conclusion:

ACR caused marked alterations in animal behaviour and early mortality level, it elicited a significant increase in serum AST and ALT, and a significant decrease of total protein, albumin and globulin levels, these biochemical changes coincide with histopathological alteration in liver tissues. The expression level of both CYP2E1 gene protein and mRNA were significantly down regulated. A significant increase in the number of DNA single strand breaks was evident with Comet assay. All recorded changes in all studied parameters were dose-dependent. So, ACR exposure either occupationally or dietary must be restricted. In addition to, raising awareness of people about its hazards.

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Detection of Community Acquired Methicillin Resistance Staphylococcus aureus among Staphylococcus aureus isolates.

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ABSTRACT: The rates of MRSA infections in the hospital, as well as the disease in the community, have continued to rise. Staphylococcal cassette chromosome *mec (*SCC*mec)* is a variable genetic element that contains the methicillin resistance determinant, *mecA.* SCC*mec* typing is one of the most important molecular tools available for distinction between community-acquired MRSA and HA-MRSA occurring on a worldwide basis. CA-MRSA has been reported to carry the loci for Panton Valentin leukocidin (PVL) in high frequency in association with the type IV *SCCmec*. **Aim of this study was to** differentiate between HA-MRSA and CA-MRSA by detection of SCC*mec* and determination the prevalence of PVL gene among MRSA isolates. **Material &methods**: A total of 34 *Staphylococcus aureus* isolates were included in this study. Susceptibility of Staphylococci was determined by, Disc diffusion method including methicillin, oxacillin and cefoxitin discs. Penicillin Binding Protein (PBP_{2a}) Latex Agglutination test was done to detect the presence of PBP_{2a} responsible for methicillin resistance. In addition genotypic identification of MRSA was carried out by detecting *mec* gene by real time PCR. Conventional PCR was carried using different set of primers for the amplification of SCC *mec* for differentiating the HA-MRSA and CA-MRSA; moreover detection of *PVL* as virulence factor was also done. **Results**: The antibiotic sensitivity of CA-MRSA ranged from (11.76% for ceftazidime) to (47.06% for Imipenem, Erythromycin and Gentamycin); while the sensitivity of HA-MRSA ranged from (2.94% for Amoxicillin and Ampicillin/sulbactam) to (29.41% for Amikin). Out of 34 *S. aureus* strains; 26(76.47%) isolates were found to be resistant to oxacillin disc, 30(88.24%) isolates were resistant to methicillin; and all strains were resistant to cefoxcitin disc. All MRSA strains were confirmed to be methicillin resistant by detection of *mecA* gene using real time PCR. Out of 34 MRSA strains 32 (94.12%) were PBP_{2a} producer. In the present study, though, the majority (25out of 34) of our strains were not SCC mec typable, yet among the nine typable strains the six hospital strains belonged to type II and III as reported in the literature and the three CA-MRSA belonged to the novel type V reported by other workers to be associated with CA-MRSA and the only PVL positive CA MRSA strain was untypable.

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious threat to hospitalized patients globally and now represents a challenge for public health, as community-acquired infections appear to be on the increase in various regions and countries, [1,2] including North America [3], Australia [1,] Saudi Arabia [4] and Finland [5]. Rising colonization rates lead to increased infection rates in the community and in hospitals. The consequence to the health care system is longer hospital stays and greater costs. [6] Patient risks include significantly higher mortality and morbidity rates with invasive MRSA infection. [7]

MRSA strains produce penicillin binding protein2a, which possesses reduced affinities for binding to β lactam antibiotics resulting in β lactam resistance. The *mecA* gene, encoding PBP2a, is carried on a peculiar type of mobile genetic element inserted into the staphylococcal chromosome, designated staphylococcal cassette chromosome mec (*SCCmec)* elements. *SCCmec* elements typically share four characteristics: first, they carry the mec gene complex (mec) consisting of the methicillin resistance determinant mecA and its regulatory genes and insertion sequences; second, they carry the ccr gene complex (ccr) consisting of ccr genes that are responsible for the mobility of the element and its surrounding sequences; third, they have characteristic directly repeated nucleotide sequences and inverted complementary sequences at both ends; and last, they integrate into the 3_ end of an open reading frame (ORF), orfX.[8]

In *S. aureus*, three major mec complex classes have been described, and concerning the ccrAB locus, three major allotypes (ccrAB1 to ccrAB3) [9] and one sporadic allotype (ccrAB4) [10] have been identified. Recently, a new type of ccr gene complex, which consists of only one gene (ccrC) not closely, related to the ccrA or ccrB gene, was reported [11]. SCCmec carries other sequences that define the overall genetic

organization of the resistance cassette. These regions may be used as targets for typing strategies [12], and polymorphisms within these regions, particularly in the region downstream of the ccrAB genes (the J1 region), define SCCmec subtypes or variants.[13]

Based on the class of *mecA* gene complex and the type of *ccr* gene complex present, SCC*mec* cassettes are classified into seven major types. [14] The hospital-associated strains of MRSA (HA-MRSA) strains contain the larger type I, II, or III cassettes [13] , while the two smallest SCC*mec* types, SCC*mec* IV and SCC*mec* V, have been associated with community-associated MRSA (CA-MRSA) [2,15]

Clinically, CA-MRSA usually causes skin and soft tissue infection. However, it can cause serious life-threatening conditions, which in addition to necrotizing pneumonia include necrotizing fasciitis, bloodstream infection, and septic shock [16]. Reports have suggested that certain strains of CA-MRSA may be more virulent than HA-MRSA [17, 18]. The expression of Panton-Valentine leukocidin (PVL), a two-component, pore-forming, cytolytic toxin that targets mononuclear and polymorphonuclear cells and causes cell death by necrosis or apoptosis, has been strongly associated with CA-MRSA [17]. The PVL toxin consists of two synergistic proteins, LukS-PVL and LukF-PVL, encoded by the *pvl* genes *lukF* and *lukS*, which are carried on a temperate bacteriophage. [2, 19]

Clinicians are now faced with emergence of (CA-MRSA) strains that are genetically different from MRSA strains originating in the hospital. Moreover, with the recent trend and shift in epidemiology, CA-MRSA is now being found in hospitals and in some instances displacing classic (HA-MRSA). [20]

The present study aimed to differentiate between hospital acquired MRSA and community acquired MRSA by detecting different Staphylococcal Chromosomal Cassette mec types (*SCCmec*), and detecting the prevalence of Panton Valentine Leukocidin as virulence factor for CA-MRSA and determining whether its carriage could be used as a surrogate marker for CAMRSA

MATERIAL AND METHODS

I-Bacterial Isolates:

Thirty nine Staphylococcal isolates were included in this study. Data recording previous hospital admission during the 6 months ago were collected**.**

I-Identification of staphylococcal isolates [21]:

The staphylococcal isolates were identified morphologically and biochemically by standard laboratory procedures. The coagulase plasma test was performed on organisms exhibiting typical staphylococcal colony morphology to allow for discrimination of *S. aureus* from coagulase-negative staphylococci.

II-Antibiotic susceptibility testing [22]: Methicillin resistance (using Methicillin, Oxacillin and Cefoxitin) and susceptibility to different antibiotics were determined by the agar disk diffusion method.

III-Detection of Penicillin Binding Protein 2a (PBP2a) Latex Agglutination Test (Oxoid®)[23] based on the agglutination of latex particles sensitized with monoclonal antibodies against PBP2a, was used according to the manufacturer's instructions. Agglutination was visualized and was scored as positive, negative, or weakly positive.

IV- Polymerase Chain Reaction (PCR)

Staphylococcal DNA extraction: staphylococcal colonies were emulsified in 200 µl sterile distilled water to produce a heavy suspension, and heated at 100°C for 15 min. then centrifuged at 14.000 rpm for 5 min.

PCR for *mecA* gene detection was performed using Real time (TaqMan) PCR. [24]. The primers and probe used were as follows: Forward primer, 5'TGCTAAAGTTCAAAAGAGTATTTATAACAAC A 3'; Reverse primer, 5'TGTGCTTACAAGTGCTAATAATTCACC 3'; and Probe, 5' FAM-ATTATGGCTCAGGTACTGCTATCCACCCTCAAA -TAMRA 3'.The PCR mixture was prepared using TaqMan® Universal PCR Master Mix (2X) with final PCR mixture volume of 25 µl. Five µl of template DNA and 30 pmole of each primer and 7.5 pmol of probe were added to each test. A negative control was prepared by the addition of the same contents to the tube with water instead of the extract. Amplification was performed using MX3000P ™ (Stratagene) Real Time PCR System programmed to hold at 95°C for 10 min for AmpliTaq gold activation and 30 cycles of denaturation at 95°C for 15 sec and annealing and extension at 60°C for 1min with end point fluorescence detection.

PCR for detection of *SCCmec* gene and *PVL*: Polymerase chain reaction for detection of SCC*mec* and *PVL* gene was performed using genomic DNA from each MRSA isolate as template. Primers used to amplify the different SCCmec and *PVL* are listed in (Table1). **For SCCmec,** 2 sets of primers were used. The first set (Oliviera primers) is designed to type *Sccmec* (I-IV). [25] based on selected loci (A through F) upstream and downstream the *mecA* gene. Another set of primers (zhang) [26] was used for detecting SCCmec type II, III and the newly described SCC mec type V.

PCR Conditions: eight µl of DNA extract were amplified by PCR in a final volume of 25 µl using 2x

PCR master mix (Fermentas life sciences®) containing 0.05 units/µl of Taq DNA polymerase,50 picomol of each primer, PCR buffer, 2 mMMgCl2, 0.2mM of each dNTP. A negative control was prepared by the addition of the same contents to the tube with water instead of the extract. Amplification was performed in a Perkin-Elmer 9600 thermocycler. For **Oliveira primers** the cycle program was performed with an initial denaturation for 5 min at 94°C, then 35 cycles of denaturation for30 sec at 94°C, annealing for 30 sec at 53°C and extension for 1min at 72°C and final extension for 5 min**.** While for **Zhang primers**: the cycles begin with an initial denaturation step at 94°C for 5 min followed by 10 cycles of 94°C for 45 seconds, 65°C for 45 seconds, and 72°C for 1.5 min and then another 25 cycles of 94°C for 45 seconds, 55°C for 45 seconds, and 72°C for 1.5 min, and end with a final extension step at 72°C for 10 min. **For amplification of *PVL* gene [(27)]**: the cycle program was performed with an initial denaturation for 5 min at 94°C, then 35 cycles of denaturation for 40 sec at 94°C, annealing for 40 sec at 53°C and extension for 1min at 72°C and final extension for 10 min.

(Table1) primers used for amplification of *SCCmec and PVL*

Locus	**Oliveira**'s **Primers** [(25)]	Oligonucleotide sequence (5'–3')	Amplicon size (bp)	Specificity (SCC*mec* type)
A	CIF2 F2 CIF2 R2	TTCGAGTTGCTGATGAAGAAGG ATTTACCACAAGGACTACCAGC	495	I
B	KDP F1 KDP R1	AATCATCTGCCATTGGTGATGC CGAATGAAGTGAAAGAAAGTGG	284	II
C	MECI P2 MECI P3	ATCAAGACTTGCATTCAGGC GCGGTTTCAATTCACTTGTC	209	II,III
D	DCS F2 DCS R1	CATCCTATGATAGCTTGGTC CTAAATCATAGCCATGACCG	342	I,II,IV
E	RIF4 F3 RIF4 R9	GTGATTGTTCGAGATATGTGG CGCTTTATCTGTATCTATCGC	243	III
F	RIF5F10 RIF5R13	TTCTTAAGTACACGCTGAATCG GTCACAGTAATTCCATCAATGC	414	III
Zhang's Primers: [(26)]		Oligonucleotide sequence (5'-3')	Amplicon size (bp)	Specificity
Type II-F Type II-R		CGTTGAAGATGATGAAGCG CGAAATCAATGGTTAATGGACC	398	SCC*mec* II
Type III-F Type III-R		CCATATTGTGTACGATGCG CCTTAGTTGTCGTAACAGATCG	280	SCC*mec* III
Type V-F Type V-R		GAACATTGTTACTTAAATGAGCG TGAAAGTTGTACCCTTGACACC	325	SCC*mec* V
PVL primers:[(27)]		Oligonucleotide sequence (5'-3')	Amplicon size (bp)	Specificity
PVL-1 PVL -2		ATCATTAGGTAAAATGTCTGGACATGATCCA GCATCAASTGTATTGGATAGCAAAAGC	433bp	PVL

Detection of PCR products was done using 2% agarose gel stained with ethidium bromide and using of the molecular weight markers (50 bp DNA ladder (Fermentas Life Sciences).

RESULTS

1-Bacterial isolates and identification: among the 39 *Staphylococcus* isolates, 34 (87.18%) were free coagulase positive by tube coagulase test.

2-Detection of methicillin resistance by Disc diffusion method: out of 34 *S. aureus* strains; 26(76.47%) isolates were resistant to oxacillin disc, 30(88.24%) to methicillin and all strains were resistant to cefoxitin disc **(100%).**

3-Antibiotic susceptibility: According to data regarding previous hospital admission, the 34 collected strains were divided into 18 CA-MRSA& 16 HA-MRSA strain. CA-MRSA were more sensitive, the sensitivity in CA-MRSA ranged from (22.22% for ceftazidime) to (88.89% for Imipenem, Erythromycin and Gentamycin); while the sensitivity in HA MRSA ranged from (6.25% for Amoxicillin and Ampicillin/sulbactam) to (62.5% for Amikin). All MRSA were sensitive to vancomycin.

4-Detection of *mec A gene*: All the 34 strains were *mecA* gene positive. The presence of *mecA* gene detected by real time PCR was considered to be the gold standard in evaluating the 3 disc diffusion methods to identify MRSA.

5- Gene expression (PBP2a Production) among the 34 *mecA* positive : out of 34 MRSA strains 32 (94.12%) were PBP_{2a} producer while 2 (5.88%) were PBP_{2a} non producer. The 2 PBP_{2a} non producers isolates were CA MRSA.

6- SCC mec Typing:

Out Of the 16 HA-MRSA: 5 isolates were *SCCmec* type II (Two by Oliveira primers and three by zhang primers) and only one was detected as type III. While out of the 18 CA-MRSA Three isolates belonged to the newly described Type V reported to be associated with CA MRSA. The remaining 25 MRSA strains could not be typed.(Fig 1(A-E)

(A) 284bp DNA ladder 284bp (B) DNA ladder 243bp

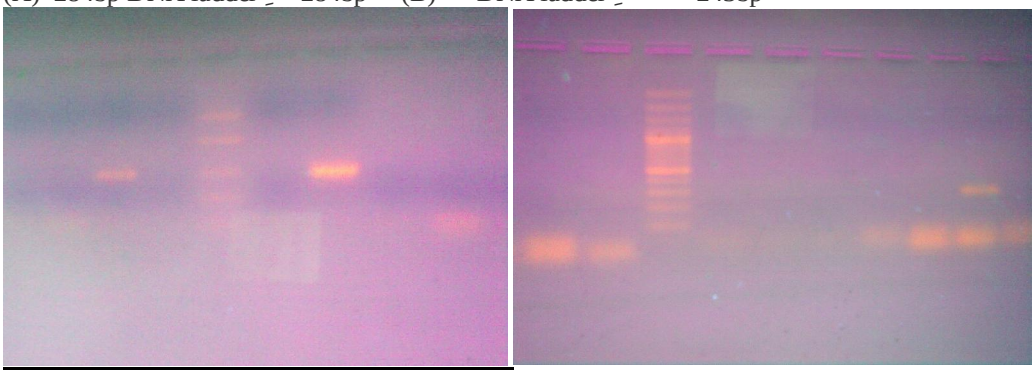


Fig (1A): Ethidium bromide stained agarose gel showing a single band of 284 bp specific for SCC mec type II at lanes 3 and 7 Lane 5 shows 50 bp DNA ladder. **Fig (1B)**: show a single band of 243 bp specific for SCC mec type III at lanes 11. Lane 5 shows 100 bp DNA ladder**.**

(C) 398 bp DNA ladder 398 bp (D) 325bp DNA ladder 325bp

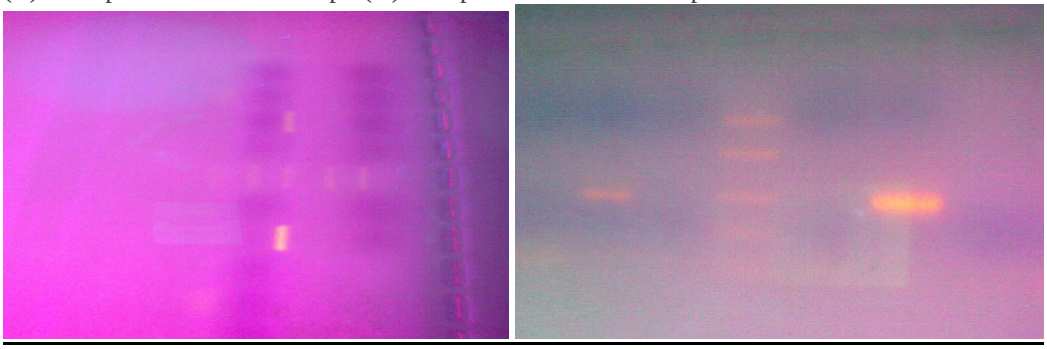
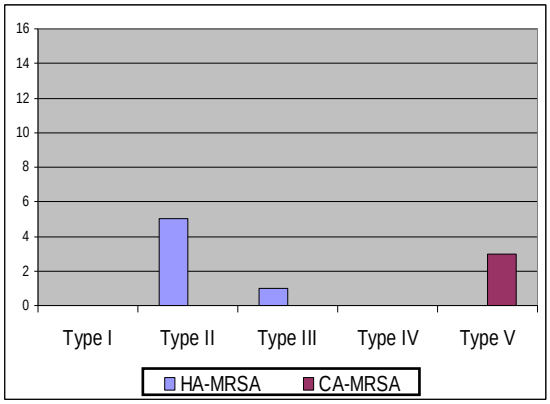


Fig (1C): Ethidium bromide stained agarose gel showing a single band of 398 bp specific for SCC mec type II at lanes 6 and 10. Lane 8 shows 50 bp DNA ladder. **Fig (1D)**: show a single band of 325bp specific for SCC mec type IV at lanes 3 and 3 Lane 12 shows 50 bp DNA ladder

7-Detection of PVL: only one strain (CA-MRSA) out of the 34 MRSA was positive for Panton Valentine leukocidin *(PVL)* by PCR. (*Fig* 2)

Fig 2: 433bp of PVL DNA ladder



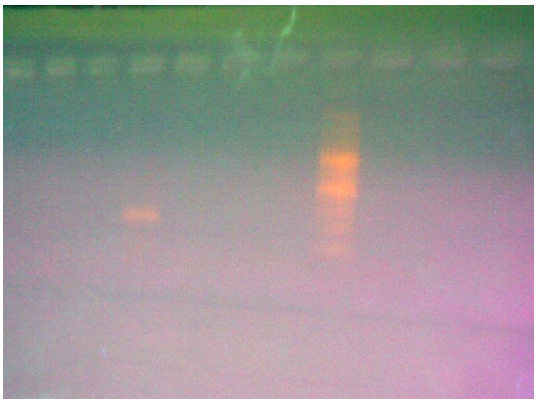


Figure 1E: SCC mec typing of the 34 MRSA

Fig 2: Ethidium bromide stained agarose gel showing a single band of 433 bp specific for PVL at lane 5. Lane 9 shows 100 bp DNA ladder

Methicillin resistance of *S.aureus* remains to be a significant problem. Rapid and accurate determination of methicillin resistance is important for initiation of appropriate antimicrobial therapy. Misdiagnosing this resistance leads to treatment failures and spread of infections with these resistant strains.

Phenotypic techniques as disk diffusion and microdilution methods are employed in routine laboratories for the detection of methicillin resistance. However, these methods are often not entirely reliable at detecting some strains that harbor the *mecA* gene. [28] Identification of the *mecA* gene remains the most reliable method of detecting MRSA isolates, however not all laboratories can include molecular biology techniques in their routine clinical practice.

In this study cefoxitin disk diffusion tests was 100% sensitive for MRSA detection. Alternatively, only (76.47%) and (88.24%) isolates were resistant to Oxacillin and methicillin respectively. These results were in accordance with those of several studies [28-30] .This means that disc diffusion testing using cefoxitin disc is far superior to most of the currently recommended phenotypic methods and is now an accepted method for the detection of MRSA by many reference groups including CLSI. [31]

Identification of MRSA, is more accurate by either directly detect the gene encoding the methicillin resistance determinant (*mecA*) or its product (PBP2a). [32] MRSA-Screen test is a rapid and simple to perform method. Many studies reported that the accuracy of MRSA screen latex agglutination test for detection of PBP2a approaches the accuracy of PCR and more accurate than susceptibility testing methods with sensitivity of 97%-100% and a specificity of 97%-99.1% [33,34]. In this work, only two (5.88%) PBP2a non producer isolates (false negative) were identified. Other authors [35, 36] reported that false-negative results may occur with MRSA isolates with low oxacillin MICs (4 or 8 µg/ml) due to production of smaller amounts of PBP_{2a} or the failure to express the gene phenotypically.

MRSA infection can be categorized into 2 distinct groups: HA-MRSA and CA-MRSA. The community isolates are distinctly different from the hospital strains both epidemiologically and microbiologically. [37] Both CA-MRSA and HA-MRSA are resistant to traditional anti-staphylococcal β-lactam antibiotics. However, CA-MRSA isolates tend to be more susceptible to other antibiotics (including to sulfa drugs, tetracyclines) than are HA-MRSA, [38] and their narrow spectrum of resistance is solely due to determinants harbored on genetic elements present on the SCC. [39] In this study, most of the 18 CA-MRSA isolates were susceptible to tetracyclines (83.3%) Erythromycin (88.9%), Gentamycin (88.9%), co-trimoxazole (77.8%), Ofloxacin (83.3%). On the other hand, the 16 HA- MRSA showed a wide spectrum of drug resistance ranging from 81.25% (ofloxacin, gentamycin and tetracyclines) to 68.7 % (erythromycin) and 56.2% (co-trimoxazole). Comparable results were obtained by various authors. [40, 41] Kaplan [40] noted that most of the CA-MRSA isolates are susceptible to most antibiotics, while Anbumani *et al*., [41] reported that 250 MRSA isolated from different clinical specimens were multi-drug resistant.

SCC*mec* typing is one of the most important molecular tools available for understanding the

epidemiology and strain relatedness of MRSA. [26] In the present study, 2 sets of primers were used in an attempt to type our MRSA strains. The first set was chosen according to Oliviera typing scheme designed to type *Sccmec* (I-IV), [25] and using six primer pairs only 3 of the 16 HA MRSA were typable by Oliviera primers. Two belonged SCC mec type II (amplified locus B) and one SCC mec type III. (amplified locus F), and none of the 18 CA MRSA could be typed by this scheme. As Oliveira's assay has limitations in detecting the newly described SCC*mec* type V, misclassifying them as type III, while failing to discriminate type IV into subtypes a, b, c, and d [26] Also, because of difficulties in assay optimization another trial of typing the SCC mec was carried using Zhang set of primers unique and specific for SCC*mec* types. So, another 3 out of 16 HA MRSA were classified as SCCmec type II, moreover another three belonged to SCCmec type V among 18 CA MRSA. So using both typing methods only 9 out of the 34 MRSA were typable. Similar data were noticed by others. Oliviera *et al*, [25] reported that, 8% of their isolates were non-typeable for SCCmec by their primers used in this study. Also, Shore *et al* [42] used also two typing methods the first for amplification of the *ccr* and *mec* genes, and the second method of Oliveira and de Lencastre, used in the present study and (50%) out of their 172 isolates harbored two apparently different SCC*mec* elements when tested by both typing methods. They suggested that PCR amplification and sequencing of the entire SCC*mec* element was necessary for the complete characterization of the SCC*mec* elements harbored by isolates with ambiguous multiplex patterns.

The differentiation between the typical HA-MRSA and CA-MRSA strains based on epidemiologic definitions becomes difficult, along with molecular distinction based on the (SCCmec) type is beginning to blur. [43] In the present study, though , the majority (25out of 34)of our strains were not *SCC mec* typable, yet among the nine typable strains the six hospital strains belonged to type II and III as reported in the literature and the three CA-MRSA belonged to the novel type V reported by other workers to be associated with CA-MRSA

CA-MRSA has been reported to carry the loci for PVL in high frequency, and to be associated with the type IV (*SCCmec*). [44] In the present study, the only PVL positive CA MRSA was untypable. This was contrary to that noted by Berglund *etal* [45] who detected PVL genes in 66% of the CA-MRSA isolates. However, Holms et al revealed that the PVL genes are carried by a relatively low number (1.6%) of *S. aureus* isolates from their clinical laboratories, indicating an unequal distribution of the genes encoding PVL among their strains. [46] In addition, the overall proportion of MRSA isolates carrying *pvl* was 1.8% among the 1,389 MRSA isolated from Ireland. 7.5% of these isolates were CA-MRSA strains, of which only 6.7% carried *pvl* genes and the carriage of *pvl* was not restricted to CA-MRSA. [47] Similarly, 78% of CA-MRSA isolates referred to a central reference facility were *pvl* negative and 25% of *pvl*-positive isolates in this group were HA. Additionally, Ko *et al*, [48] was not able to detect *PVL* gene, in any of their MRSA isolates. Moreover, Kim *et al*, [49] reported that none of their Korean CA-MRSA isolates contained the PVL genes. These findings agree with reports that carriage of *pvl* cannot be used as a sole marker for CA-MRSA. [47]

In the present study , the combination of SCC mec typing , in addition to the detection of PV leucocidin was not sufficient to discriminate between HA and CA MRSA due to the near absence of PVL among the CA MRSA and the limited capacity of the *SCC mec* typing methods among our strains.

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Comparative Antioxidant Activity Study of Some Edible Plants Used Spices in Egypt.

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ABSTRACT: There is an increasing demand for natural antioxidants to replace synthetic additives in the food industry. Many spices have been shown to impart an antioxidative effect in foods. The spices are defined as dry plant material that is normally added to food to impart flavor. Methanol, methanol and water (1:1), water (37°C), water (100°C) extracts of ten edible plants (spices: cumin, chili, pepper, nutmeg, garlic, cloves, ginger, coriander, onion and thyme) were tested as extractants of total polyphenols, antioxidant activities. Antioxidant activities of the extracts were evaluated by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay and a β-carotene bleaching assay. Methanol extract of cloves showed the highest total phenolics content (171.8 mg garlic acid equivalents/100 g dry weight cloves powder). Total antioxidant activity of the ten spices determined by radical scavenging (DPPH) were ranged from (26.19-85.31%). The antioxidant activity by β-carotene-lenoleic acid were ranged from (36.55-85.43%). Methanol extract of cloves showed the highest antioxidant activity by DPPH of β-carotene-linoleic acid methods were (85.31, 85.43% respectively).

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Key Words: cumin, chili, papper, nutmeg, garlic, cloves, ginger, coriander, onion, thyme, total phenolics, antioxidant activity, solvent.

INTRODUCTION:

Oxidation of lipids which occurs during raw material storage, processing, heat treatment and further storage of final products is one of the basic processes causing rancidity in food products leading to their deterioration. A large number of experimental studies indicate that lipid oxidation products, called free radicals, can harm healthy cells, create harmful molecules, and contribute to the degenerative processes related to aging and diseases e.g. cancer, cardiovascular disease, and neurodegenerative disorders, such as Alzheimer's disease (Croft, 1999, Lemberkovics *et al.* 2002, Sami 1995, Shon *et al.* 2003). The antioxidants are now known to play an important role in protection against disorders caused by oxidant damage. The term antioxidants refers to compounds that can inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reaction (Velioglu *et al*. 1998) and which can thus prevent or repair damage done to the body's cells by oxygen. They act in one or more of the following ways: reducing agents, free scavengers, potential complexers of pro-oxidant metals and quenchers of singlet oxygen (Hudson 1990). There is an increasing demand for natural antioxidants to replace synthetic additives in the food industry. Natural antioxidant substances are presumed to be safe since they occur in plant foods. Natural antioxidants occur in all higher plants and in all parts of the plant (Wood, bark, stems, pods, leaves, fruit, roots, flowers, pollen and seeds). The antioxidant compounds of higher plants have been demonstrated in vitro experiments to protect against oxidation damage by inhibiting or quenching free radicals and reactive oxygen species. The roles of these compounds are potential antioxidants can be inferred by their similarity to synthetic antioxidants of related structure (Larson 1988).

In the current study we evaluated the antioxidant activity of extracts of ten edible plants species with two methods based on different mechanisms. The objective of this study was to investigate the effect of extracting solvents on the yield of polyphenols and antioxidant activity.

MATERIALS AND METHODS:

Plant materials:

The dried plant parts from following species were studied: - Cuminum cymimum, Capsicum annuum, Piper nigrum, Myristica fragrans, Allium sativum Syzgium aromaticum, Eugenia caryophyllis, Zingiber officinale, Coriandrum sativum, Allium cepa and Thymus vulgaris. They were purchased from a local market.

Preparation of Crude Plant Extract:

Five grams of sample powders were mixed with 20 ml of either (1) methanol, (2) methanol and water (1:1), (3) water in a rotary shaker at 37°C for 12h or (4) water boiled (100°C) in water bath with stirring for 12h. The mixtures were then filtered (Whatman No. 1). The filtrates were then concentrated in a rotary evaporator until dried.

Total Phenolics Determination:

The total phenolics content of the ten samples were determined by the Folin-Ciocalteau method (Duarte-Almeida *et al*. 2006). Brielly, 0.5 ml diluted extract solution was shaken for 1 min 100 µl of Folin-Ciocalteau reagent and 6ml of distilled water. The mixture was shaken and 2ml of 15% Na_2CO_3 were added and shaken once again for 30s. Finally, the solution was brought up to 10ml by adding distilled water. After 1.5h, the absorbance at 750 nm, was evaluated using a spectrophotometer. The results were expressed as gallic acid equivalents.

Determination of DPPH-radical scavenging capacity:

The antioxidant activity of plant extracts and the standards was assessed on basis of the radical scavenging effect of the stable DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical was determined by the method described by (Shimada *et al*. 1992). Briefly, 1ml of extract and 5ml of freshly prepared 0.1mM DPPH methanolic solution were thoroughly mixed and kept in the dark for 60 min. The absorbance of the reaction mixture at 517 nm was measured with a spectrophotometer. The blank was prepared by replacing the extract with 1ml of methanol or methanol + water (1:1) or water. The percentage of free radical scavenging activity was calculated as follows:-

Scavenging activity (%) = [1 – (A sample/A blank)] x 100

Determination of antioxidant activity by β-carotene bleaching method:

This experiment was carried out by the method of Emmons *et al*. (1999). β-carotene (5mg) was dissolved in 50ml of chloroform, and 3ml was added to 40 mg of Linoleic acid and 400 mg of tween 40. Chloroform was then removed in a rotary vacuum evaporator. Distilled water (100ml) was added and mixed well. Aliquots (3ml) of the β-carotene/linoleic acid emulsion were mixed with 40µl of sample solution and incubated in a water bath at 50°C. Oxidation of the emulsion was monitored spectrophotometrically by measuring absorbance at 470nm over a 60 min period. Control samples contained 40µl of solvent in place of the extract. The antioxidant activity is expressed as percent inhibition relative to the control after a 60 min incubation using the following equation:-

$AA = (DR_C – DR_S)/DR_C$

Where AA is the antioxidant activity.

DR_C is the degradation rate of the control = (In(a/b)/60)

DR_S is the degradation rate in the presence of the sample = (In(a/b)/60).

(a) is the initial absorbance at time 0 min and (b) is the absorbance at 60 min.

RESULTS:

The antioxidant activity of plants is mainly contributed by the active compounds present in them. The total polyphenol and antioxidant activity of ten edible plants (spices: cumin, chili, pepper, nutmeg, garlic, cloves, ginger, coriander, onion and thyme) were determined. The total polyphenols content in ten plants extracts determined by the Folin-Ciocalteu method and were ranged from (12.5 – 171.8 mg/100g dry.wt) (Table 1). The total polyphenol content of cumin water (37°C) extract was the highest one (60.4 mg/100g dry weight) when expressed against the polyphenol of methanol or methanol and water (1:1) or water (100°C) extracts. Although the methanol and water (1:1) extract of nut meg, chili or garlic was the highest level of polyphenol (112.9, 63.9, 27.6 mg/100g dry weight) respectively and the water (100°C) extract of coriander, pepper or onion extract was the highest one (70.6, 62.7, 32.4 mg/100g dry weight) respectively.

Table (1): Total phenolics in plant extracts.

Plant	Scientific name	Total phenolics (mg/100g dry weight)			
		Methanol extract	Methanol + water extract	Water (37°C) extract	Water (100°C) extract
Cumin	*Cuminum Cyminum*	45.7	54.3	60.4	56.5
Chili	*Capsicum annuum*	40.4	63.9	39.6	53.4
Pepper	*Piper nigrum*	38.7	51.5	46.3	62.7
Nut meg	*Myristica fragrans*	38.6	112.9	102.4	50.4
Garlic	*Allium Sativum*	12.5	27.6	23.9	25.3
Cloves	*Syzgium aromaticum*	171.8	164.0	160.9	166.6
Ginger	*Zingiber officinale*	94.8	87.6	67.4	76.5
Coriander	*Coriandrum Sativum*	42.5	34.2	39.6	70.6
Onion	*Allium Cepa*	18.4	28.6	24.8	32.4
Thyme	*Thymus Vulgaris*	22.8	15.5	15.6	14.2

The methanol extract of cloves, ginger, thyme had the highest recovery rate against the other extracts (171.8, 94.8, 22.8 mg gallic equivalent/100g dry wet) respectively.

Table (2): Antioxidant activity of ten methanol, methanol and water (1:1), water (37°C) and water (100°C) extract with DPPH method.

Plant sample	DPPH free radical scavenging activity %			
	Methanol extract	Methanol + water extract	Water (37°C) extract	Water (100°C) extract
Cumin	60.60	60.50	59.40	57.38
Chili	60.95	59.00	58.30	58.45
Pepper	56.97	57.52	56.60	56.00
Nut meg	53.30	62.27	58.21	52.49
Garlic	54.71	53.80	54.00	26.39
Cloves	85.31	82.00	56.74	77.22
Ginger	43.50	29.93	28.15	26.19
Coriander	50.39	55.02	55.96	53.42
Onion	56.04	55.31	55.32	51.17
Thyme	63.24	62.32	62.26	61.87

In the DPPH assay, the ability of the investigated extracts to act as donors of hydrogen atoms or electrons in transformation of DPPH radical into its reduced form DPPH-H was investigated.

In the present study we have evaluated the free radical scavenger activity of methanol, methanol and water (1:a), water (37°C), water (100°c) extracts of cumin, chili, pepper, nutmeg, garlic, cloves, ginger, coriander, onion, thyme. The methanolic extract of cumin, chili, garlic, Cloves, Onion, Thyme (60.6, 60.95, 54.7, 85.31, 43.5, 56.04, 63.24 respectively) were higher than other extracts but the methanol and water (1:1) extract was the higher one for papper, nutmeg (57.52, 62.27 respectively). water (37°c) extract of coriander (55.96%) was the highest one.

The β-carotene bleaching method is usually used to evaluate the antioxidant activity of compounds in emulsions accompanied with the coupled oxidation of β-carotene and linoleic acid.

Table (3) shows the antioxidant activity coefficients (AAC) of ten plants extracts by methanol or methanol and water (1:1) or water (37°C) or water (100°C). The antioxidant activity of water extract (37°C) for cumin, chili, garlic, coriander, thyme (81.8, 76.75, 82.69, 81.77, 79.38% respectively) wee higher than methanol or methanol and water or water (100°c) extracts. however, methanol extract of nutmeg, cloves, ginger, onion (67.39, 85.43, 78.50, 82.97 respectively) gives the highest activity. The water (100°C) extract of pepper (67.84%) was the highest one.

Table (3): Antioxidant activity of ten methanol, methanol and water, water (37°C) and water (100°) extract with β-catotene bleaching method.

Plant sample	β-carotene bleaching AAC			
	Methanol extract	Methanol + water extract	Water (37°C) extract	Water (100°C) extract
Cumin	56.13	63.30	81.80	51.54
Chili	69.58	55.39	76.75	59.62
Pepper	60.32	42.71	64.38	67.84
Nut meg	67.39	51.20	55.04	49.53
Garlic	64.36	69.70	82.69	71.30
Cloves	85.43	64.70	71.70	36.55
Ginger	78.50	61.10	69.57	49.96
Coriander	66.60	58.50	81.77	68.56
Onion	82.97	64.89	72.02	57.45
Thyme	76.86	67.30	79.38	63.05

ACC – the antioxidant activity coefficient calculated (as described in experimental part).

DISCUSSION:

The antioxidant capacity and total phenolics content of fruit, vegetables, herbs and spices have received increasing attention recently for their potential role in prevention of human diseases as well as in food quality improvement (Kamatha *e al*. 2004 and Tangkanakul *et al*. 2009). Spices and Herbs are one of the most important targets to search for natural antioxidants from the point of view of safety. In the study we checked the effects of solvents on antioxidant activity and phenolic content of ten plants (cumin, chili, pepper, nutmeg, garlic, cloves ginger, coriander, onion, thyme) extracts.

Extraction is critical to the recovery of antioxidant phytochemicals. The extraction yield depends on solvent, time and temperature of extraction as well as the chemical nature of the sample. Under the same time and temperature conditions, the solvent used and the chemical property of sample are the two most important factors (Shimada *et al*. 1992). In our experiment methanol extract of cloves, ginger, thyme had the highest recovery rate against the other extracts. These results were higher than the results of Badami *et al*. (2007) who found that the water extract of coriander, ginger, pepper were (3.35, 2.85, 3.84 mg/gm of gallic acid equivalent) respectively.

The antioxidant activity of plant extracts vary with assay methods (Sun and Ho 2005). Therefore a single assay may be in adequate (Yen *et al*., 2005). For this reason we cross checked antioxidant activities of extracts of ten spices with two antioxidant activity assays based on different mechanisms, namely DPPH assay based on electron-transfer reaction and β-carotene bleaching assay based on hydrogen atom transfer reaction. Many studies indicated that only polar extracts of plants showed effective antioxidant activity and some researches further proved that moderate polarity extracts are more potent even if their total antioxidant recovery from the plant is not high (Wangensteen *et al*. 2004).

Methanol appears to perform best in extracting polar compounds such as phenolics, flavonoids and other polar material in cereals (Watanabe 1998). In the present study methanol extract of cloves, ginger, onion showed the highest antioxidant activity by DPPH assay and β-carotene bleaching assay. But the water extract (37°C) of coriander was the highest level of antioxidant activity by the two methods. These results were higher than the results of (Badami *et al*. 2007).

CONCLUSION:

The extracting solvent affected total phenolics content and antioxidant activity of ten edible plants (spices: cumin, chili, pepper, nutmeg, garlic, cloves, ginger, coriander, onion and thyme) extracts. The ten plants represent a good sources of natural antioxidants and they could be considered as useful sources of materials for human health .

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One Country, Two Systems: The Dualistic Land Tenure System in Sierra Leone, and the Need for Reform

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Abstract: Several studies have indicated a strong link between poverty and insecure land tenure. In Sierra Leone like other former British colonies, two separate land tenure systems exist: an imposed British tenure in the western area, and a customary system in the rest of the country. Whilst the former allows freehold tenure, the latter does not. Seventy-five percent of its population are rural, and invariably depends on agriculture for livelihood sustainability. Statistics also show that women who form the bulk of this population are involved in food production. One of the reasons identified why the country is unable to feed its population is the existence of the customary system. In this work, we discussed the two land tenure systems in the country, and analyzed the shortcomings of the customary tenure in detail. Data were mainly desktop literature. We looked at similar cases elsewhere and drew our conclusions. . We discovered that the customary system is not only discriminating against women, and other citizens (from other parts of the country), but discourages investment in agriculture and other land uses in rural areas. It is therefore a threat to food security and rural development in general. It also provokes tension between citizens from the western area, and those from the rest of the country. If the Poverty Reduction Strategy Paper, VISION 2025 and the Millennium Development Goals are to be realized, it is important that authorities step up and reform this customary system, and encourage more access to land, say freehold tenure.

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1. Introduction

For the millions of the world's poor living in rural areas and depending on agriculture, livestock or forests for their livelihoods, secured access to productive land is critical. This is because it does not only reduce their vulnerability to hunger and poverty (FAO, 2005:1), but influence their capacity to invest in productive activities and at the same time managing the resources sustainably (IFAD, 2008).In these areas, those with insecure tenure rights are the landless or near landless. They are poor, most marginalized and vulnerable. Such a strong correlation between poverty and land tenure insecurity, and improving access to land and poverty reduction have been confirmed at micro-level research on the causes and dynamics of rural poverty around the globe (FAO, 2007:1; Musahara, 2006).

Land tenure describes all the relations established among people to determine their various rights in the use of land (Kuhnen,). These rights may be fixed by custom or law and are often explained as a complex or bundle of rights which, together, constitute the property, i.e. the right to control an economic good, in this case, land. Thus the recognition of the importance of land tenure issues for the long term growth, poverty reduction, peace and civic empowerment has been echoed in a number of policy documents. Generally, the nature of tenure a landowner has in land would affect his ability or otherwise to use the land in the most economic manner possible. In addition, the duration of the rights would affect the type of investments one would be inclined to make on the land. And as Place et al (1994, 19) observed, land tenure security only exists when an individual perceives that he or she has rights to a piece of land on a continuous basis, devoid of imposition or outside interference, as well as ability to reap the benefits of labor and capital invested in that land either in use or any other transaction to such as transfer to another holder.

Research in many African countries identified, among other things, insecure land tenure as a major reason for the set-back in its economic development. For example, a British Royal Commission set up to

investigate and recommend ways for the promotion of economic development in some of its former colonies in Africa identified the customary land tenure system as key factor retarding economic development in the region (Mugambwa ,2007).

Customary land tenure is defined by the local social organization, including its culture. It is usually based on hierarchical principles of kingship, indigenousness and gender, with women excluded (Djire, 2005b).Several FAO and World Bank studies on customary land tenure in Africa and elsewhere indicate that customary land tenure is based on the needs of a simple subsistence economy and the social relationships that are associated with land use in such an economy .It is of a communal nature and as such has no commercial value (Mugambwa, 2007; Word Bank, 2009). For sound agricultural development to be realized, a farmer needs tenure which makes available to him a secured parcel of land and system of farming where he has an indefeasible title as will encourage him to invest in labor, and profits in the development of his farm, and also enable him to use it as a collateral for financial credit (Mugambwa, 2007; Swynnerton, 1955). And if rural areas are to develop, and the Millennium Development Goals achieved in poor countries, there is need to have a reform of customary system. This has been the focus of many research and advocacy by such bodies as The World Bank, The United Nations Economic Commission on Africa ,African Union Commission (Kagwanja,2003), to name but a few.

Sierra Leone, one of the poorest countries in Africa has a dualistic land tenure system: a free-hold and customary land tenure systems. But unlike other countries which have made strides in reforming their customary system, no major development has been made in that direction. And if some of the aims of the Poverty Reduction Strategy Paper (GOSL, 2003a) and the Millennium Development Goals are to be achieved, it is believed reforming the customary system would play a pivotal role. This paper is therefore calling for the need to have a reform of this system, and if possible, harmonization of the entire land tenure in the country. There is need to develop a land policy that would promote agricultural and economic development as well as poverty eradication in the country. The challenge for the reform is to have a balance between the needs of the population and their traditional sector on one hand, and the developing cash economy on the other.

2. Materials and Methods

2.1 Study Area

Sierra Leone is found on the west coast of Africa, between latitudes 7 and 10 degrees north, and 10 and 14 degrees west. With an area extent of 73,326 square kilometers, its people depend heavily on natural resources, including land. Statistics show that seventy percent of the 4.8 million people live in abject poverty; three-quarters of which are rural and depend on the land for livelihood sustainability. Twenty-six percent of the poor are described as “food poor” and cannot afford a basic diet (SSL, 2004; GOSL, 2005). Subsistence agricultural and “small scale” or artisanal mining are done in the poorer districts where 8 out of 10 are considered poor. The subsistence agriculture, otherwise known as the “hoe and cutlass” aims to grow food for consumption, with little or nothing left for commercial purposes.

Characteristic of its poverty include, poor housing, high illiteracy rate, poor health, and high infant and maternal mortality, insufficient food, limited access to clean water, and lack of money. With the help of the World Bank, and other international bodies, a Poverty Reduction Strategy document was produced, which serve as a blue-print for addressing the poverty situation in the country. The issue of land is a stake in alleviating the poverty menace. However, more than five years after the document, there is little sign that the majority of the poor, who form the bulk of the population and live in the rural areas, would ever get out of the poverty trap. This is because, among others, the existing customary tenure that in the rural areas in the country.

This research is therefore trying to discuss the land tenure system in the country; in particular, we focus on the demerits of the customary tenure, which is seen as providing an unfavorable climate for poverty reduction, and rural development as a whole. We have not dealt with the mechanism of how this customary system should be reformed nor have we addressed the issue of land titling and agrarian reforms in general .We have basically identified the bottlenecks this system poses as opposed to the freehold system.

2.2 Data source

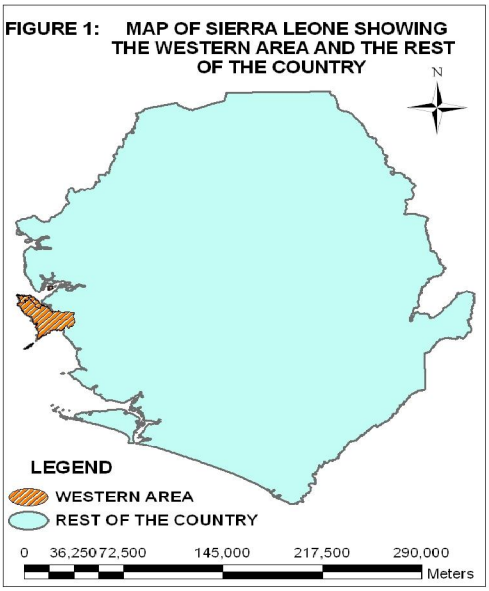
The main source of data is desktop literature on the land tenure system in Sierra Leone, and other related literature.

3. The evolution of land tenure in Sierra Leone

3.1 First British influence

As a former British colony, Sierra Leone has a dualistic land tenure system: an imposed external system practiced in the western area, including the capital city, and a peasant proprietorship in the remaining parts of the country (Figure 1). A local ruler, King Naimbana offered the British crown in 1788 several square kilometers of land for the

establishment of a free community of British subjects. This land was later converted in 1808 to the base for freed slaves (GOSL, 1933). Thenceforth, to the end of the 19th.century, the crown extended domain through treaties with tribal Kings.



3.2 Colony and protectorate divided

In 1901, Ordinance 33 defined the main administrative division of the country into two: the colony, which included the capital and the lands bought by the crown in the western area, and the protectorate, the lands of the rest of the country. The colony, under direct British rule, adopted English law where private ownership of land (for both sexes) is recognized, to date. In the Protectorate, land was acquired through a concession Ordinance 8 of 1902. Beyond this, any policy development was in the hands of indigenous tenure systems. All Acts and Regulations passed for the administration and management of land and its resources were totally different between the western area or colony (under direct British rule), and the Protectorate, which was under an indirect rule (Renner-Thomas, 2004).

3.3 Land laws of the western area/colony

Despite the ultimate advantages of freehold ownership of land by both sexes in the Western Area, and registration of such rights, the Colonial authorities seemed somewhat heavy handed in their powers over such areas as "unoccupied" and "crown lands". Ordinance No.1 of 1872 was enacted to allow the Administration's acquisition of land for public purposes in the colony area. In 1898, this power was translated into "Crown land". The" Crown Land" covered a small area, compared to the needs for construction, especially in the capital, Freetown (Renner-Thomas, 2004). The administration sought to control indiscriminate claims to land and to preserve some of it. This led to the ultimate definition of "Crown Land" in No.19 Ordinance of 1960. Crown lands were considered to be:-i) those lands that were acquired by, or for the Crown, either through treaty, convention, concession, or agreement, for public use, or otherwise, and ii) lands acquired under the provisions of the Public Lands Ordinance (Turay, 2006).

In 1906, Ordinance Cap 117 established the "Unoccupied Lands (Ascertainment of Title) as well as the Registration of Instruments Ordinance (Cap 256).Registration of land titles was not recognized but a deed. This meant acquisition of land between generations of family members was done without a real right holder title. The main aim of the "unoccupied" lands Ordinance was to curb land grabbing while the provision was made to prove ownership through Statutory Declarations based on the 1835 Act of England (Turay, 2006).

Two other ordinances, the Forestry Ordinance No.8 of 1912 permitted the setting aside of Reserves on Crown Lands (Forster, 2004), while the Survey Ordinance 20 of 1927 allowed surveying an individual landowner's or occupier's property in preparation for registration and conveyance. Statutory declarations also came into existence (for proof of title to land) through declaration under oath showing how the title was possessed. Such a declaration of ownership was to be supported by two witnesses and a survey plan for registration under the registration of Instruments Act 256 of 1960 (GOSL, 2003b).

3.4 The Protectorate (Provinces) land laws

Apart from Ordinance 1 of 1872 which made provision for the Crown to acquire land for public purposes, the Protectorate was largely left to develop its own land policies based on communal tenure. The Concessions Ordinance 8 of 1902 enabled land acquisition for such use as plantation agriculture, construction for central government and other such uses (Turay, 2006).

In 1903, the Protectorate Court jurisdiction Ordinance was enacted to empower the local rulers, known as Paramount Chiefs to decide all cases of land dispute except those between two Paramount chiefs. Ordinance 16 0f 1905 solved the problem of non-native infiltration to Provincial lands. This ordinance conferred the vesting of land to the *tribal authority*, with the exception of land under the

concessions ordinance and land for public purposes. In general, lands in the protectorate are meant for the indigenes and exclude "non-natives", including those from the western area of the country. Immigrants are however allowed to hold land on lease. The central government could also possess land through the Paramount Chief and or *tribal authority (*GOSL, 2005*)*. This is made possible by the Protectorate Law (Amendment) Ordinance No.15 of 1961, which states that the expression "non-native" shall not include the government. Government can therefore, at any time, anywhere possess land for its use (Turay, 2006).
Indigenes are not allowed to own land in their own names. Plots of land are given to heads of families for agricultural purposes. Such lands could be transferred from one member of the family to another. Female members of the family are however not allowed to hold land on behalf of their families, even where they are the head, mother or widows.

From the aforementioned, the following comes out clearly on the land tenure issue in Sierra Leone.
-two separate land tenure systems exist: a freehold system (in the western area and capital city), and a customary tenure (in the rest of the country).
-no freehold under customary system;
-the customary system discriminates against both non-natives and women.

4. Discussion of Implications

Like other former British colonies (example, Nigeria, Ghana, Zimbabwe, Uganda, Papua New Guinea etc.), the customary land tenure system has been identified as an impediment to the socio-economic development of developing countries. In most of these countries, reforms were made after civil strides (Kenya), tribal wars (Uganda), land invasions (South Africa, Zimbabwe) and political tensions. As Mugambwa (2007); Adams (1995) noted, such reform involves the individualization or distribution of property or rights in land for the benefit of the landless, tenants and farm laborers. Ezigbalike and Cyprian (1999) went further to assert that it goes beyond redistribution. It also involves such rural development initiatives and facilities as improved farm credit, cooperatives for farm-input supply and marketing, and extension services which would promote the productive use of the land reallocated.
Whilst there has not been any major resentment (for example, tribal conflict, land invasion/grabbing etc) against the customary tenure system in Sierra Leone, it is however characterized by issues that provoke tension and social instability. The following have been identified as the main bottlenecks of the customary system in Sierra Leone, and which calls for the reform.

4.1 Lack of freehold means little or no empowerment

As seen above, the main characteristic of customary tenure is the lack of freehold of land by individuals. Tenure is vested in the hands of local authorities and their subjects, with the former determining who should own land as determined by societal rules and regulations. There is no permanent hold on such lands though, as official titling is completely absent, and so cannot be used as an economic good. We argue in this paper that this tenure be reformed into a freehold.

While critics of such a reform would argue that changing the customary tenure to individual freehold would destroy the cultural ties of the society which are the very fibre holding them together, the reality is that it is only the free hold tenure that empowers indigenes, and owning such lands amongst kinsmen raises the social status of individuals.
In Sierra Leone, the customary tenure emphasizes cultural valves at the expense of economic and financial gains of the land. To empower the rural populace and help develop the rural areas for example, capital in the form of land is a necessity. Reforming the customary system to a freehold increases the value of land, as the economic forces of demand and supply would determine its price. This introduces a land market where land could be sold, leased, mortgaged, exchanged or otherwise deal with commercially. The existence of land market facilitates access to land ownership with financial means, including women and immigrants. Moreover, trade in land can produce financial resources for vendors. Land is also a source of social status and bargaining power (Doss et al 2007).
In urban areas, those with land are guaranteed more revenue which could enable them to engage in more productive ventures such as setting up private businesses and partnership with landless non-investors. In the same vain, lands become collateral in such areas. Land owners could also mortgage or lease their lands to gain access to cash for other ventures. Ownership to land is in itself liberation from poverty, as its guarantees investment in whatever measure.

4.2 Threat to food security and poverty alleviation

As the primary source of sustenance, land is used by members of society for livelihood sustainability (James, 1987). Generally, access to land is a critical factor in the eradication of food insecurity and rural poverty (FAO, 2003:2). Inadequate access to land and insecure tenure of

those rights often result in entrenched poverty and lead to rural underdevelopment and food insecurity (FAO 2003:2). More secured access to land enhances productivity and improve the financial status of the rural populace. And as spelt out in Pillar Two of its Poverty Reduction Strategy Paper, the aim of the food security strategy in Sierra Leone is to empower poor rural and urban households to improve the food they consume, and encouraging farm families to produce more. Government aims at supporting small-scale subsistence farmers who dominate agriculture, to diversify and increase production, improving crop storage, feeder roads and market access; and encourage private agriculture investment.

However, from every indication, the customary tenure has made such dreams impossible. The country still imports more than eighty percent of the staple food it consumes, and the populace is still entrenched in abject poverty, in both the rural and urban areas. In terms of investment and agricultural development, the customary tenure does not provide adequate incentives for growth in the agricultural sector. And with the country relying on imported food to feed its population, the need for large scale investment (into the agricultural sector) by the private sector is seen as a way towards achieving self-food sufficiency, poverty alleviation and increasing the country's GDP. One fact that has emerged in recent times is that there has been a significant reduction in the agricultural population (MOAFS, 2007; 2008). One of the reasons could be as a result of lack of secured tenure in land. Indigenes, in whose care land parcels are, also charge renters high rent on such lands. Renters refuse to pay, citing little profit after particular planting season. This results in abandoning agricultural activities all-together. Whilst holders of such lands abandon the countryside for the city, investors with the cash stay away from the land because of lack of tenure. Urbanization is therefore taking its toll on the major cities, with unemployment figures swelling, and the number of people going down the poverty line also increasing.

4.3 Discourages investment and job creation

The lack of investment outside of the capital such as large-scale commercial agricultural projects, and other business ventures can partly be attributed to the problems associated with acquiring land for such economic activities. For the country to develop, it demands investment from both indigenous and private entrepreneurs. This is enshrined in the Millennium Development Goals, and VISION 2025- to attain a competitive private sector-led economic development with effective indigenous participation. Investment outside of the capital has a number of positives to it. For example, it would reduce unemployment among the indigenes. Associated with this is the fact that it would reverse the rural-urban drift which has been observed in the country in the last 10 to 20 years, prompted by lack of jobs and underdevelopment in the rural areas.

Under the customary system also, landowners do not gain access to financial institutions because such lands are not accepted as collateral for example, agricultural loans. With the bulk of the rural population that is poor, access to loan encourages agricultural expansion and a move from the subsistence type of agriculture into a commercial one. This empowers the rural populace and reduces poverty. Improved access to land enables a family to increase household income by producing surpluses for sale, and help improve the ability of a household to access credit. Secure access to land provides a valuable safety net as a source of shelter, food and income in terms of hardship, and a family land can be the last available resort in the instance of disaster (FAO, 2003:2).

The customary tenure thus discourages large-scale foreign investors, especially if such investors find it difficult to obtain large parcels of land during a single transaction. The lack of investment in an area such as agriculture means the country would rely on imported food to feed its growing population. This in itself is unhealthy to the country's economy.

Aside agriculture, investment in other land uses such as ecotourism, education and skill training centres, and shopping areas, also address unemployment, increase literacy and empower the populace, and attract other line-investment from overseas investors (FAO, 2002).

4.4 Gender bias

Women do the bulk of the farm work in Sierra Leone. They are not only involved in food production, but also in knowledge dissemination and skills related to food, agriculture, and management of natural resources (MAFFS, 2004.Unfortunately,they are not entitled to land under the customary tenure system. This is because places outside the capital practice patrilineal descent and has a patrilinear residential system. Women are therefore unprotected and disadvantaged in areas having customary land tenure. It is therefore believed that security of tenure in the rural areas could empower them, and at the same time encouraging those with capital to invest on the land, and adopt sustainable farming practices with better care of other resources.

Another characteristic of rural women in Sierra Leone is the fact that they are the heads of their households. The situation is as a result of most men

abandoning rural areas to find employment in the city or mining areas. In their absence, the lack of makes it impossible for women to put to use family plots without the consent of the husband or male members of the family. This is because women in Sierra Leone are considered to be property of either their husbands, or parents. And in the event of divorce or death of a husband (or head of family), female members and their dependants do not lay claim to land. They and the land are considered property of relatives, husbands or male subjects and are transferable.

4.5 Discrimination against other citizens

The customary tenure prevents other citizens (*non-natives*) from owning land outside of their places of birth, apart from the western area. This could be described as social injustice. This could lead into conflict and alienation. Clearly, access to land (for all citizens) plays an important role in social harmony and the overall development of the country. Indigenes from other parts of the country are possible investors, in not only the agricultural sector but social services, infrastructure, and education. Reform would provide a more equitable distribution of land resources and reduce social injustices.

5. Conclusion

Like many developing countries that depend on agriculture, Sierra Leone needs to reform its customary system to allow individual freehold not only for *non-natives*, but women as well. Food insecurity and agrarian reform are closely linked, and until individualized access to land is ensured for the majority of the rural people, the reduction of poverty, elimination of hunger, and rural development in general, will not be achieved. The poor, who make up the bulk of the population and live in rural area, should have access to land, in order to improve their lives. Free and unhindered access to land encourages investment into rural areas and other urban settings outside of the capital. This would help develop these areas and hence would not only bring in employment to the youths, but also minimize the rural-urban drift that has strained the limited resources in the cities, in particular the capital. And if the Poverty Reduction Strategy Paper and VISION 2025 are to be achieved, it is necessary for policy makers to make swift response to reform the customary tenure system into one of more access to land.

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